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THE PROTEIN SYNTHESIS RESPONSE IN RAT SKELETAL MUSCLE FOLLOWING ACUTE SPRINT OR ENDURANCE RUNNING AS MEASURED BY THE INCORPORATION OF L-(4,5 ^{3}H)-LEUCINE INTO PROTEIN AND LEUCYL-tRNA

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled THE PROTEIN SYNTHESIS RESPONSE IN RAT SKELETAL MUSCLE FOLLOWING ACUTE SPRINT OR ENDURANCE RUNNING AS MEASURED BY THE INCORPORATION OF L-(4,5 ^{3}H)-LEUCINE INTO PROTEIN AND LEUCYL-tRNA submitted by JACQUES A. DALLAIRE in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

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John G. Wilkinson

External Examiner

Date May 8, 1980

University of Alberta

DEDICATION

To my loving wife Fern, who supported and stood by me during my seemingly endless years as a graduate student, and to the new joy in my life, Dominique.

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ABSTRACT

In order to study the post-exercise response of protein synthesis to different acute exercise loads, 54 male rats ran at either 30m/min for 30 minutes or 90m/min, 10:20s work:rest, for 30 minutes on each of two consecutive days. Animals were sacrificed and soleus and plantaris muscles were excised either pre-exercise or at 0, 12, 24, 36, 48, 60, or 72 hours post exercise. *In vivo* incorporation of L-(4,5 ^{3}H)-leucine was measured (dpm/mg protein) in the whole homogenate and in three subcellular fractions. The results indicate that the response of protein synthesis to acute exercise may not follow the same time-course pattern in all animals. It would appear that the protein synthetic response to sprint exercise is delayed with respect to that of endurance exercise (*ie.* endurance response range - 12 to 36h; sprint response range - 24 to 48h). It is possible that this delay is intensity related. As well, the response of leucyl-tRNA to acute exercise would indicate that part of the mechanism by which the training effect is built involves modification of the *translation* step of protein synthesis.

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I. INTRODUCTION

It is generally accepted that mammalian skeletal muscle contains two major classes of fiber types which can be histochemically distinguished on the basis of differences in myofibrillar ATPase activity (Dubowitz and Pearse, 1960; Edstrom and Nystrom, 1969; Gollnick *et al.*, 1972a, 1972b). Further differentiation of these two basic skeletal muscle fiber types into oxidative and non-oxidative categories has been achieved as a result of their response to staining procedures designed to demonstrate oxidative potential (NADH Tetrazolium Reductase activity - Novikoff *et al.*, 1961). As a result, the nomenclature proposed by Peter and co-workers (1972) has gained popularity and is routinely utilized to distinguish fibers in animals as Fast-Glycolytic (FG), Fast-Oxidative-Glycolytic (FOG), or Slow-Oxidative (SO). In addition, electromyographic evidence from single motor units (Henneman and Olson, 1965; Grimby and Hannerz, 1968; Tanji and Kato, 1973) as well as studies of glycogen depletion patterns in animals and man (Armstrong *et al.*, 1974, 1975; Edgerton and Simpson, 1969; Piehl, 1974) substantiate the existence of certain muscle fiber pools which have different metabolic, structural and functional characteristics. These pools may be preferentially recruited to best meet the work demands. (Hannerz and Grimby, 1973).

It is well documented that as a result of physical training (chronic exercise stress), skeletal muscle is capable of adapting to different physiological demands by

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altering its structure and function. This capacity for change has been demonstrated in contractile elements as a result of sprint types of work (myofibrillar protein - Gordon *et al.*, 1967; Jaweed *et al.*, 1974) and in enzyme activity in intermediary metabolism as a result of endurance types of work (Barnard *et al.*, 1970, 1971; Holloszy *et al.*, 1973, 1975a, 1975b). However, even though these long term adaptive responses in skeletal muscle are relatively well known, virtually no information exists concerning the acute mechanisms involved in these adaptive responses.

As these demonstrated changes in muscle fibers involve nucleic acids and proteins, the purposes of this study were as follows:

- To determine the acute effects of sprint and endurance exercise on protein synthesis in whole muscle homogenate, myofibrillar-nuclear, mitochondrial, and soluble fractions of soleus and plantaris muscles as measured by the incorporation into protein of the radionuclide, L-(4,5 ^{3}H)-leucine.
- To determine the possible role of transfer RNA (tRNA) in the protein synthetic response as measured by the specific activity of L-(4,5 ^{3}H)-leucyl-tRNA isolated from the soleus and plantaris muscles and,
- to examine the time-course (0 to 72 hours post-exercise) over which the acute protein synthetic response to sprint or endurance exercise stress occurs.

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II. METHODOLOGY

A. ANIMAL CARE

Seventy male Wistar rats were obtained at approximately five weeks of age (100 - 125g) from the Charles River farms in Newfield, N.J. (LAI=COX(WI), origin - National Institute of Health Colonies). The animals were housed in pairs in self-cleaning cages in an air conditioned room at 22°C and the day/night cycle was adjusted to maintain the lighted period from 6pm to 6am for the remainder of the experiment. The rats were fed a regular diet of *Purina Rat Chow* (23% crude protein) and given water *ad libitum*. All animals were handled, food and water were replenished and soiled papers changed daily. The cages were washed and sterilized and the rats weighed weekly. After six days of orientation to the laboratory, all animals were subjectively screened for treadmill running ability during the first two days of the treadmill acclimation period. Of the 70 animals obtained, 54 were judged able to perform the running task. These animals were then randomly assigned to either a control, sprint, or endurance acclimated groups.

B. ACCLIMATION PROTOCOL

All exercises were performed on a pre-calibrated motor-driven rodent treadmill (Quinton MDL 2A) which was divided into ten compartments (9.5 cm wide and 48.0 cm long) with a shock grid at the back of each. Acclimation was

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Table I. The acclimation protocol utilized to bring animals from the *sprint* group to a criterion running level.

Acclimation Day	Bout	Speed(m/min)	Grade(%)	Duration(10:20s)
1	am	10	0	3min
1	pm	15	0	3min
2	am	20	5	3min
2	pm	20	5	3min
3	am	20	5	6
3	pm	20	5	6
4	am	40	5	8
4	pm	50	5	8
5	am	50	10	10
5	pm	50	10	12
6	am	60	10	12
6	pm	60	10	12
7	am	70	10	16
7	pm	70	10	16
8	am	80	10	16
8	pm	80	10	20
9	am	90	10	20
9	pm	90	10	20
10	am	90	10	24
10	pm	90	10	24

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Table II. The acclimation protocol utilized to bring animals from the *endurance* group to a criterion running level.

Acclimation Day	Bout	Speed(m/min)	Grade(%)	Duration(min)
1	am	10	0	3
1	pm	15	0	3
2	am	20	5	3
2	pm	20	5	3
3	am	20	5	4
3	pm	20	5	4
4	am	30	5	5
4	pm	30	5	5
5	am	30	10	6
5	pm	30	10	6
6	am	30	10	7
6	pm	30	10	7
7	am	30	10	8
7	pm	30	10	8
8	am	30	10	9
8	pm	30	10	9
9	am	30	10	10
9	pm	30	10	10
10	am	30	10	12
10	pm	30	10	12

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Table III. Aerobic (endurance) and anaerobic (sprint) experimental groups and post-exercise sacrifice times chosen for observation. (SED.C. - sedentary control; ACC.C. - acclimated control)

			SACRIFICE TIME (HRS)							
	SED.C.	ACC.C.	0	12	24	36	48	60	72	
EXERCISE TREATMENT	AE.	3*	3	3	3	3	3	3	3	3
	AN.		3	4	3	4	3	4	3	3

* - Number of Animals per Cell

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performed at approximately 9:00 hours and 14:00 hours, four days per week (*ie.* Mon., Tues., Thurs., and Fri.).

The acclimation schedule for the animals assigned to the sprint group is outlined in Table I. This acclimation procedure was designed to progressively load the animals until they were able to complete 24 repetitions of 10:20s (work:rest) at 90 m/min and 10% grade.

The acclimation schedule for the animals assigned to the endurance group is outlined in Table II. This procedure was designed to progressively load the animals until they were capable of performing work of a continuous nature at a speed of 30 m/min and a grade of 10% for twelve minutes.

Following the final session of the acclimation period, the animals received a 72 hour rest after which they were subjected to an exercise bout according to the protocol described in the following section.

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C. PERFORMANCE PROTOCOL

Following the acclimation period, the animals from each of the treatment groups (*ie.* control, sprint and endurance acclimated) were randomly assigned to each group as outlined in Table III.

The animals of the sprint acclimated group performed work at the speed, grade, and work:rest ratio as in the acclimation period. However, the repetions were increased to 60 and the exercise was completed on each of two consecutive days (24 hours between sessions).

The animals of the endurance acclimated group ran at the same speed and grade as in the acclimation period but the duration was increased to 30 minutes and the exercise was completed on each of two days as outlined above. In this way, the distance covered by the animals of both exercise groups was equal.

Following the performance exercise bouts, animals from the 12 to 72 hour sacrifice groups were returned to their cages until their scheduled sacrifice times and were allowed access to food and water *ad libitum*.

D. TISSUE HANDLING

At selected times following the completion of their final performance bouts (Table III) the animals were sacrificed by decapitation (small animal guillotine), exsanguinated and a sample of approximately 5.0 ml of whole blood was collected for separation of plasma by centrifugation (clinical centrifuge - Fischer Scientific Co.) and subsequent radioactive counting as outlined in the section 'Radionuclide Counting Procedures'. Ten minutes prior to their respective sacrifice times, all animals received an intravenous injection (saphenous vein) of L-(4,5 ^{3}H)-leucine (25 μCi per 100 g body weight; (S.A.- 37.0 GBq/mmole leucine - obtained from Amersham Corp.)) in a physiological saline solution. The muscles from these animals were used to determine labelled leucine incorporation into protein fractions and leucyl-tRNA

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specific activity. Since the processing of the tissue required 5 minutes (pilot work), a ten minute pre-sacrifice injection time was chosen so that the duration of *in vivo* incorporation of the labelled amino acid was 15 minutes from the time of injection to the time of tissue processing.

After exsanguination, the right and left soleus and plantaris muscles were quickly isolated, excised and trimmed of visible connective tissue and fat. The muscles of the left legs were then weighed, quick frozen in isopentane pre-chilled in liquid nitrogen, and stored at -70°C in pre-cooled containers until later analysis for leucyl-tRNA specific activity could be performed. The muscles of the right legs were placed in a pre-cooled petri dish and bathed with an ice cold (4°C) physiological saline solution prior to immediate processing for leucine incorporation into protein.

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E. BIOCHEMICAL PROCEDURES

MUSCLE FRACTIONATION

The right leg soleus and plantaris muscles were then blotted, weighed and placed in a chilled homogenation solution consisting of 0.32 M sucrose, 10.0 mM leucine and 5.0 mM EDTA (pH 7.4) to a volume of 3.0 ml/100 mg wet weight muscle. Each muscle was then minced with chilled scissors and homogenized in a *Polytron* homogenizer (Brinkman Comp.) by one 5-second burst at a rheostat setting of 5 to yield an even suspension.

TOTAL HOMOGENATE

To 0.5 ml of the homogenate, 0.1 ml of 10% sodium dodecyl sulphate (SDS) was added and left to dissolve for 10 min at room temperature. The proteins in each fraction were then precipitated by addition of 3.0 ml of 20% trichloracetic acid (TCA), collected by centrifugation for 10 min at 1000 x g in a clinical centrifuge (Fischer Scientific Co.) and washed and re-centrifuged 3 times with 5.0 ml of a 10% TCA wash containing 10.0 mM leucine. Following the final centrifugation, the remaining pellet was dissolved (36°C) in 0.5 ml of 1 N NaOH, diluted by an equal volume of distilled water, and aliquots were taken in duplicate for protein determination and radioactive counting.

MYOFIBRILLAR - NUCLEAR FRACTION

The remaining homogenate was centrifuged in a refrigerated high speed centrifuge (Ivan Sorval model RC2B with an HB4 rotor) at 2500 x g for 10 minutes. The supernatant was decanted and collected for isolation of the mitochondrial and soluble fractions and the remaining pellet was re-suspended in homogenation buffer to the same volume as the original. From this suspension, 3.0 ml of solution was transferred to another tube and re-centrifuged at 2500 x g for 10 minutes. The supernatant was decanted and discarded and the remaining pellet was washed and centrifuged twice with the same volume of homogenation solution. Following the final centrifugation, the remaining pellet was dissolved

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(36°C) in 0.5 ml of 1 N NaOH, diluted by an equal volume of distilled water and aliquots were taken in duplicate for protein determination and radioactive counting.

MITOCHONDRIAL FRACTION

The initial supernatant from the myofibrillar-nuclear preparation was transferred to 15 ml *Corex* tubes and centrifuged at 12,000 x g for 15 minutes. The supernatant was decanted and collected as the soluble fraction. The remaining pellet was washed (washes were discarded) and re-centrifuged three times with 5.0 ml of a 10% TCA wash containing 10.0 mM leucine. After the final centrifugation, the remaining pellet was dissolved (36°C) in 0.2 ml of 1 N NaOH, diluted with an equal volume of distilled water and aliquots were taken in duplicate for protein determination and radioactive counting.

SOLUBLE FRACTION

To the supernatant from the mitochondrial preparation was added 10% SDS, to a final concentration of 0.5%, for 10 min at room temperature. To this solution, 100%(w/v) TCA was added to a final concentration of 20 % and the sample left to stand on ice for 15 minutes. Those proteins which were precititated by TCA were isolated by centrifugation in a clinical centrifuge at 1000 x g for 10 minutes and the remaining pellet was washed and re-centrifuged three times with 5.0 ml of a 10% TCA wash containing 10 mM leucine. After the final centrifugation the remaining pellet was dissolved (36°C) in 0.2 ml of 1 N NaOH, diluted with an

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equal volume of distilled water and aliquots were taken in duplicate for protein determination and radioactive counting.

F. LEUCYL - tRNA ISOLATION

Soleus and plantaris muscles from the left legs were removed from the freezer (-70°C) and crushed in liquid nitrogen by means of a mortar and pestle. The powdered muscle was then transferred to a 15.0 ml *Corex* tube and mixed, using a *Teflon* stirring rod, with 10 volumes of a homogenation buffer containing 0.09 M sodium acetate, 1.0% heparin and 1.0% SDS (pH 5.1). After 5 minutes at room temperature the homogenate was centrifuged at 15,000 x g for 15 min in a refrigerated high speed centrifuge (Ivan Sorval model RC2B with an HB4 rotor), the supernatant collected into another tube and the precipitate discarded.

Isolation of nucleic acids was performed using the phenol-sodium acetate mixture described by Allen *et al.* (1969), with the following modifications. Phenol was added (2 x volume) to the supernatant and the mixture was well agitated (*Vortex*) and left to stand for 10 min at room temperature. The solution was mixed again at the end of the 10 min period and an equal volume of chloroform was added to the phenol. After further mixing, the solution was centrifuged at 15,000 x g for 5 min and the bottom layer was drawn off by Pasteur pipet, discarded and an equal volume of chloroform was added to the top layer. The solution was

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again mixed and centrifuged at 15,000 x g for 5 min and the wash step with chloroform was repeated. Following the final wash, the top layer was carefully removed and transferred to a 12.0 ml conical tube where 2 volumes of ice cold ethanol were added and left to stand in the freezer (-20°C) for 48 hours. The extracted RNA was then collected by centrifugation at 5,000 x g for 10 minutes and the precipitate in ethanol was dissolved in 0.2 ml of a mixture containing 2.5 M NaCl and 20% Na acetate (pH 5.1), and the resulting solution left in the cold (4°C) until isolation of tRNA by column chromatography using a modification of the system of Martin *et al.* (1977). In place of the Sephadex G100 used by Martin *et al.* (1977), a Sephacryl 200 Ultrafine (Pharmacia Fine Chemicals - Sweden) resin bed was previously equilibrated with 0.1 M sodium phosphate buffer (pH 7.0) and the sample of RNA was eluted with the same medium. A sample chromatograph of the RNA extracted from skeletal muscle is presented in Appendix B (figure 18), as is an example of the specific activity calculation for tRNA (Appendix B, Table XIV). The eluent was collected in a fraction collector (Fisher Scientific Co.) and the absorbance measured (Zeiss Spectrophotometer model M 4 Q III) to determine the ratio of absorbance at 260 / 280nm. The radioactivity of each fraction in the tRNA peak absorbance range was assessed by the same procedure as described for the protein associated tritiated activity. The relative specific activity of tRNA was expressed as disintegrations per minute (dpm) / optical

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density (OD) unit at a wavelength of 260nm.

G. RADIONUCLIDE COUNTING PROCEDURES AND PROTEIN DETERMINATION

Aliquots were taken in duplicate for the determination of L-(4,5 ^{3}H)-leucine specific activity and counted in 22.0 ml borosilicate disposible scintillation vials in a Beckman LS 200 liquid scintillation counter.

In order to determine circulating levels of the radionuclide, 20 µl aliquots of serum were counted with 10.0 ml of a scintillation cocktail (*Aquasol II* - New England Nuclear Corp.) and 0.75 ml of distilled water. Results were expressed as total disintegrations per minute (dpm/20 µl volume).

In order to assess protein associated tritiated activity, 0.3 ml aliquots of the dissolved protein from total homogenate and myofibrillar-nuclear fractions and 0.1 ml aliquots from mitochondrial and soluble fractions were counted with 10.0 ml of *Aquasol II* and 0.75 ml of distilled water. The results were expressed as specific activity (S.A.) in dpm / mg of protein in each sample.

In order to measure leucyl-tRNA associated tritiated activity, 1.0 ml of eluted RNA from the chromatographic separation described previously was counted with 10.0 ml of *Aquasol II*. Results were expressed as specific activity in dpm / optical density (OD) unit at a wavelength of 260nm.

Counting efficiency was established using an internal

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standard technique (Appendix D, Table XXI) and verified using the external standard ratio.

Protein determinations were made in duplicate using a modified Biuret technique (Appendix D) and bovine serum albumin (Sigma Chemical Corp.) as a standard.

H. EXPERIMENTAL PROTOCOL AND DATA ANALYSIS

The experimental treatment of the seventeen groups of animals used is described in Table III.

Animals designated as sedentary controls (SED.C.) performed no required daily exercise.

Animals in aerobic and anaerobic acclimated groups were trained under endurance (AE.ACC.) and sprint (AN.ACC.) acclimation programs respectively, but, were sacrificed without having received any acute experimental exercise treatment.

Animals in aerobic and anaerobic groups (AE. and AN. 0, 12, 24, 36, 48, 60 and 72 hours) were sacrificed at 12 hour intervals following an acute bout of either endurance (AE.) or sprint (AN.) treadmill running.

Animals received an intravenous injection of L-(4,5 ^{3}H)-leucine 10 minutes prior to sacrifice at which time soleus and plantaris muscles were removed, homogenized, and fractionated by the differential centrifugation techniques previously described to yield total homogenate(TOT), myofibrillar-nuclear(MYO), mitochondrial(MIT), and soluble(SOL) fractions.

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Since the main purpose of this study was to determine the acute effects of sprint or endurance exercise on protein metabolism in skeletal muscle, the protein synthesis and leucyl-tRNA metabolism data were graphed to identify any trends or tendencies. Initially, the Chi Square test of Bartlett for Homogeneity of Variance (Winer, 1962) was applied to the protein synthesis data and where no difference in variance was shown, a Two-Way Analysis of Variance (ANOVA) Fixed Effect Model with unequal observations per cell (D.E.R.S. ANOV25 Program) was used to compare main effects (A, B) and interaction (AB) of the data from each fraction. The main effects referred to are:

Factor A - Exercise Treatment (Aerobic, Anaerobic)

Factor B - Time (hrs)(SED.C., ACC.C., and 0, 12, 24, 36, 48, 60 and 72 hours after the final exercise). Post Hoc procedures, if necessary, involved Scheffé's multiple comparisons of main effects (1959).

Significant differences for all statistical applications in this study, unless otherwise specified, were accepted at an alpha level where $P \leq 0.05$ (P is the probability that no differences exist between means).

In an attempt to check reliability of methodological technique with respect to protein determinations and radionuclide counting, a t-test (Ferguson, 1966) was applied to the results (protein concentration and radioactivity) obtained from duplicate samples of the same fraction of muscle taken from five randomly selected animals. An alpha

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level where $P \leq 0.05$ was used.

Finally, in order to ascertain whether or not any differences existed in blood ^{3}H-leucine pools in animals from each group, a Two-Way ANOVA Fixed Effect Model with unequal observations per cell was also applied to the serum radioactivity data (dpm/20µl serum). Again, an alpha level where $p \leq 0.05$ was used.

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III. RESULTS

The descriptive and statistical results are presented under three general headings: 1) Tritiated Leucine Incorporation into Protein, 2) Transfer RNA - Protein Synthesis Relationship, and 3) Methodological Reliability.

Individual data are summarized in tabular and graphic form, and pertinent data for all experimental animals is presented in Tables XIX and XX, Appendix C.

A. TRITIATED LEUCINE INCORPORATION INTO PROTEIN

In figures 1 through 16, the amount of ^{3}H-leucine incorporated into protein was plotted against the time elapsed after the acute, two-day exercise protocol and for the sedentary control and acclimated animals. In these figures, the ^{3}H-leucine incorporation was expressed as relative specific activity (S.A.) in dpm / mg of protein.

As listed in Table IV, the incorporation was measured in the proteins of whole muscle homogenate as well as in myofibrillar-nuclear, mitochondrial, and soluble fractions.

Figures 1 through 16 indicate that the relative S.A. is markedly elevated in some animals which were sacrificed hours after completion of the exercise. Although the increase was not consistent in all animals sacrificed at a specific time, it generally occurred in a specific time range after exercise. Whenever the increased incorporation was observed in one fraction, similar changes occur in other fractions.

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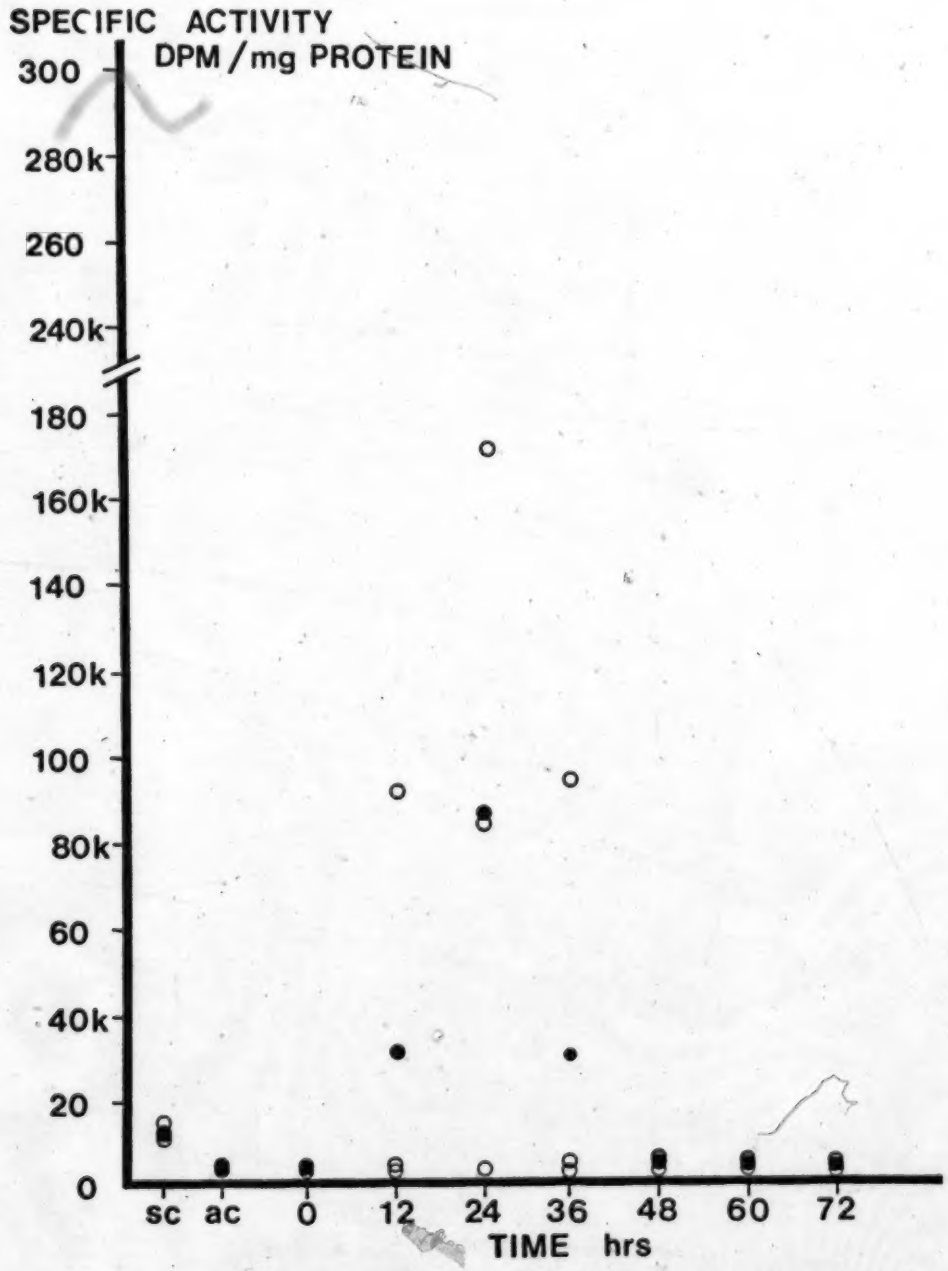

Figure 1. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the total homogenate fraction of soleus muscle from animals of the endurance group (SC-sedentary control; AC-acclimated control) (O-individual animal data;●-group mean)

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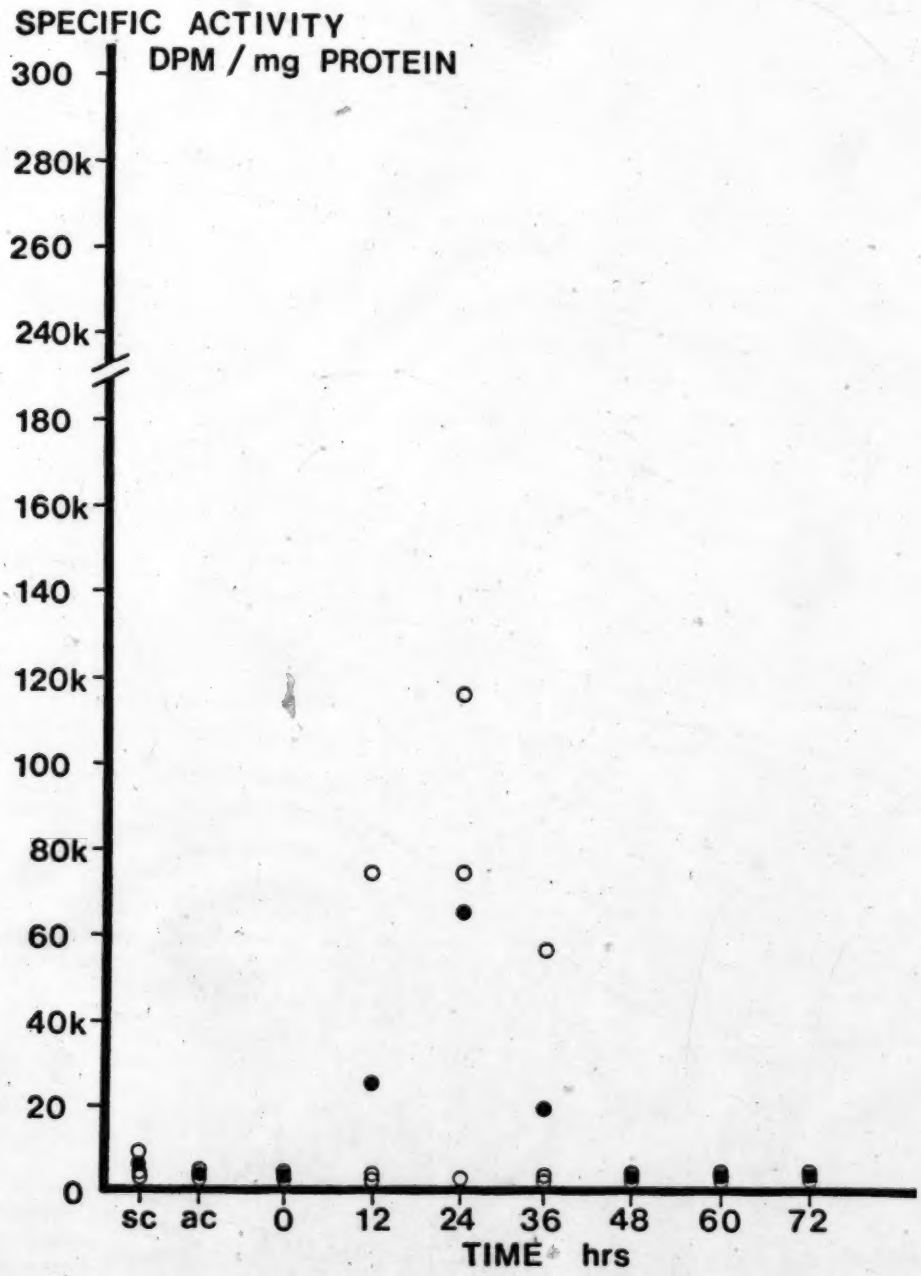

Figure 2. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the myofibrillar-nuclear fraction of soleus muscle from animals of the endurance group (SC-sedentary control; AC-acclimated control) (O-individual animal data;●-group mean)

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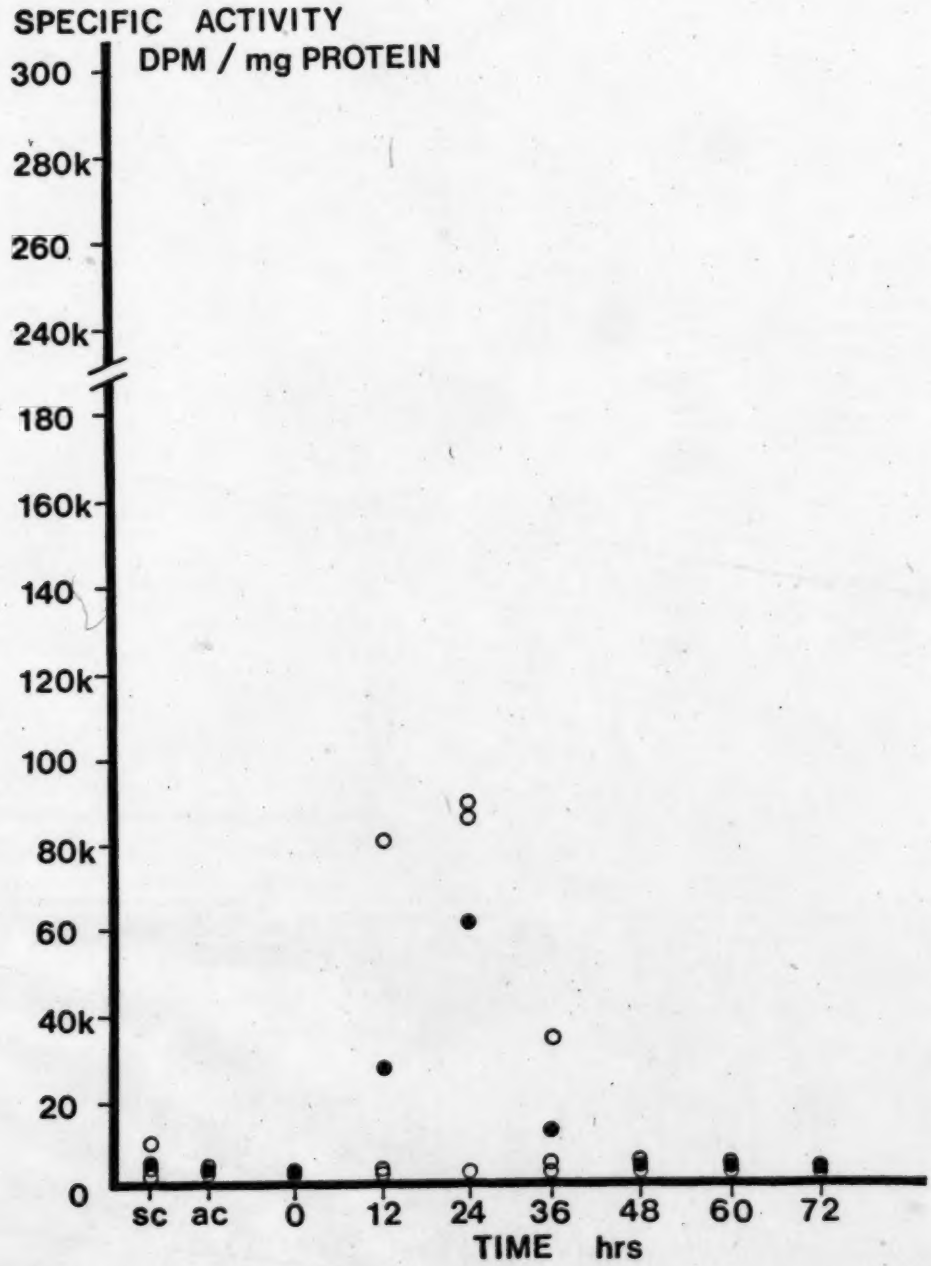

Figure 3. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the mitochondrial fraction of soleus muscle from animals of the endurance group (SC-sedentary control; AC-acclimated control) (O-individual animal data;●-group mean)

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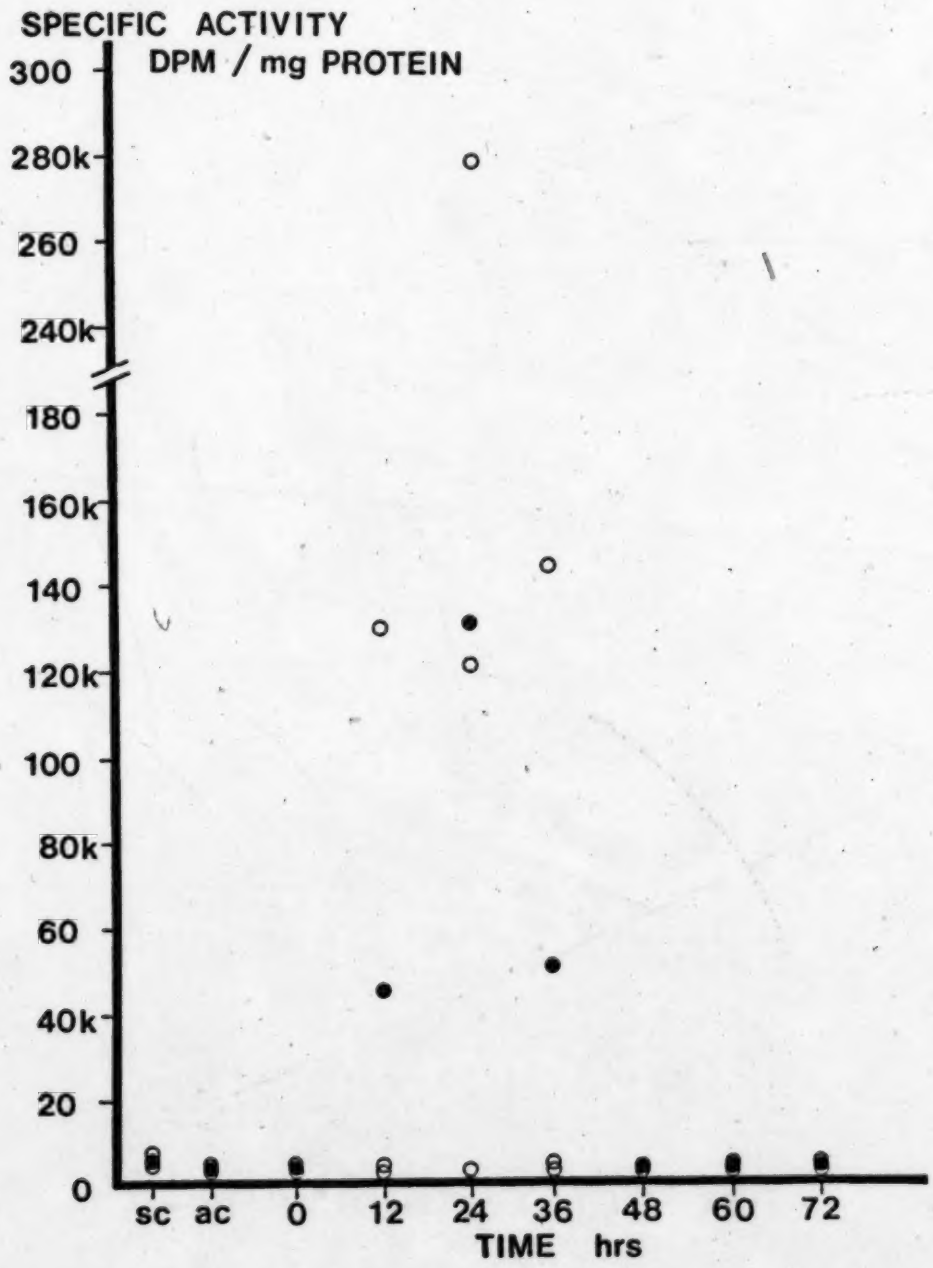

Figure 4. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the soluble fraction of soleus muscle from animals of the endurance group (SC-sedentary control; AC-acclimated control) (O-individual animal data;●-group mean)

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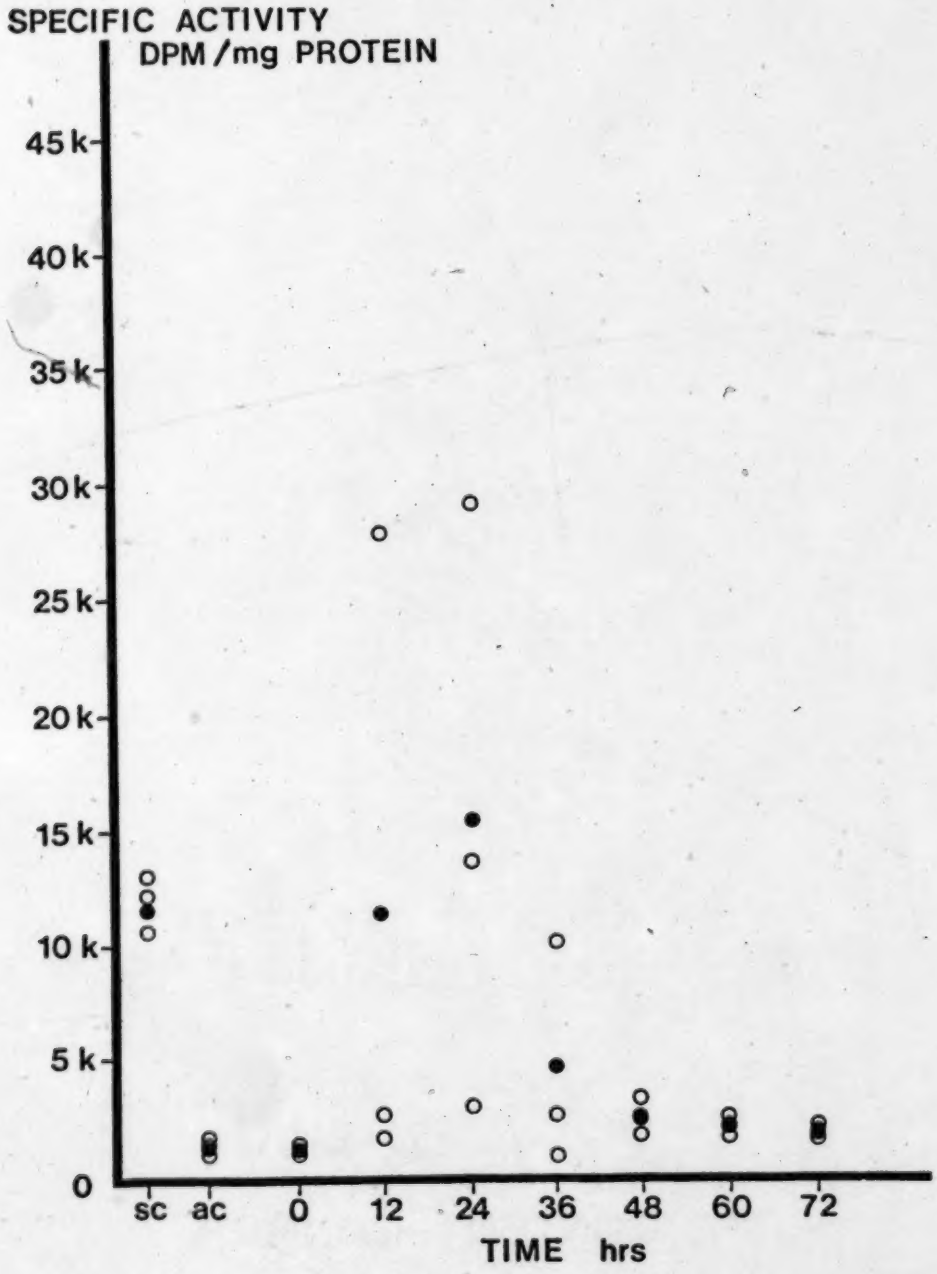

Figure 5. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the total homogenate fraction of plantaris muscle from animals of the endurance group (SC-sedentary control; AC-acclimated control)
(O-individual animal data;●-group mean)

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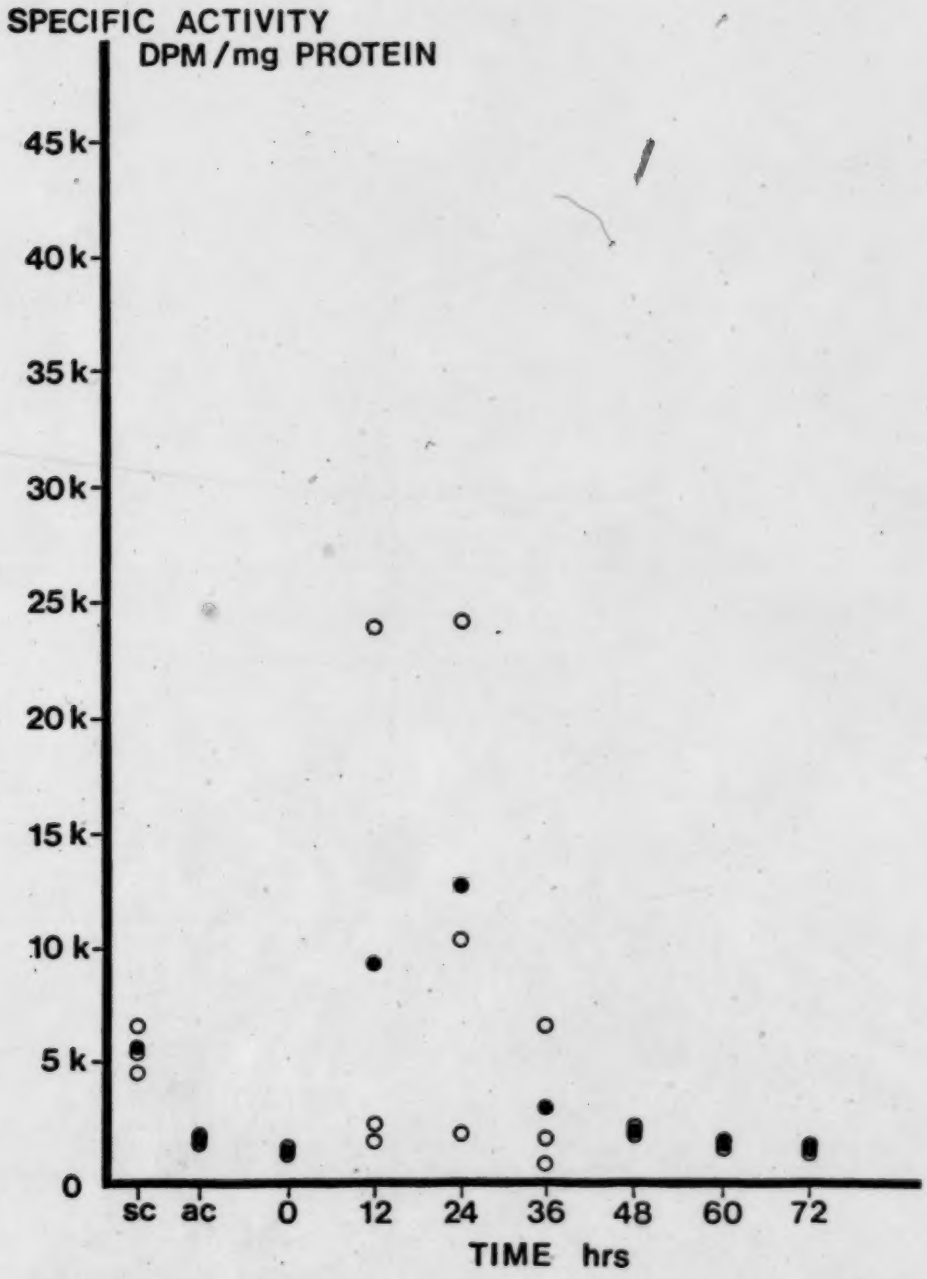

Figure 6. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the myofibrillar-nuclear fraction of plantaris muscle from animals of the endurance group (SC-sedentary control; AC-acclimated control)
(O-individual animal data;●-group mean)

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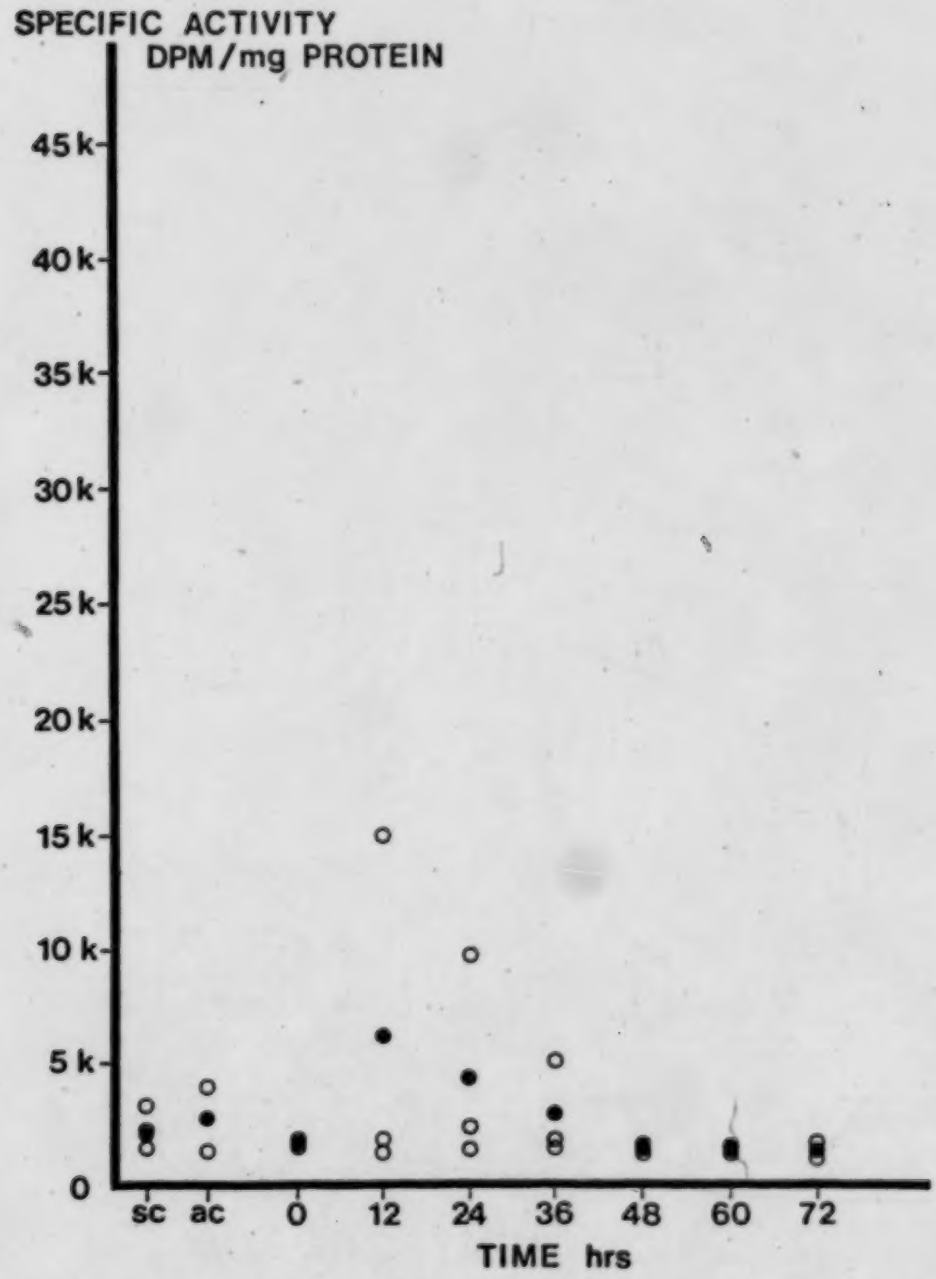

Figure 7. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the mitochondrial fraction of plantaris muscle from animals of the endurance group (SC-sedentary control; AC-acclimated control) (O-individual animal data;●-group mean)

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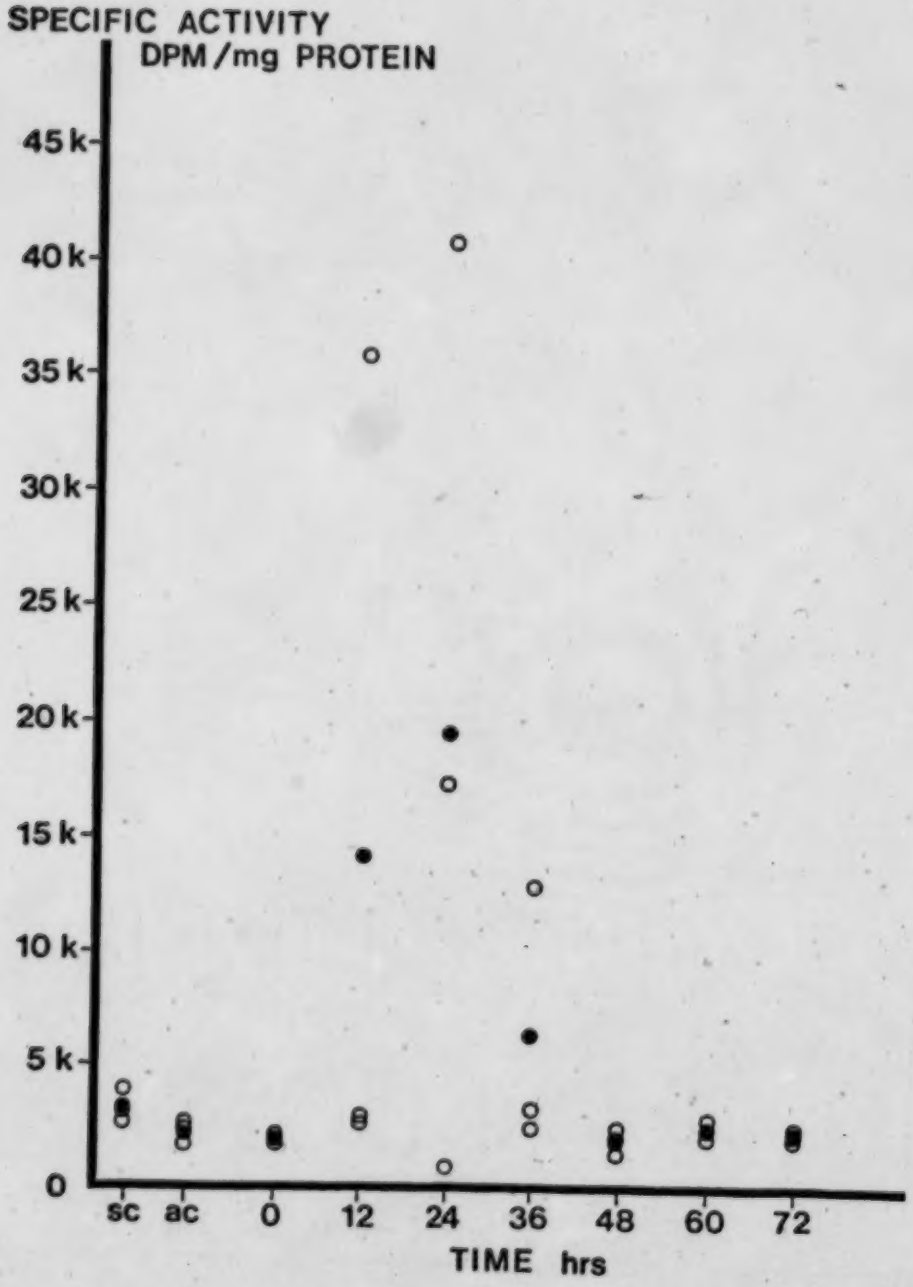

Figure 8. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the soluble fraction of plantaris muscle from animals of the endurance group (SC-sedentary control; AC-acclimated control) (O-individual animal data; ●-group mean)

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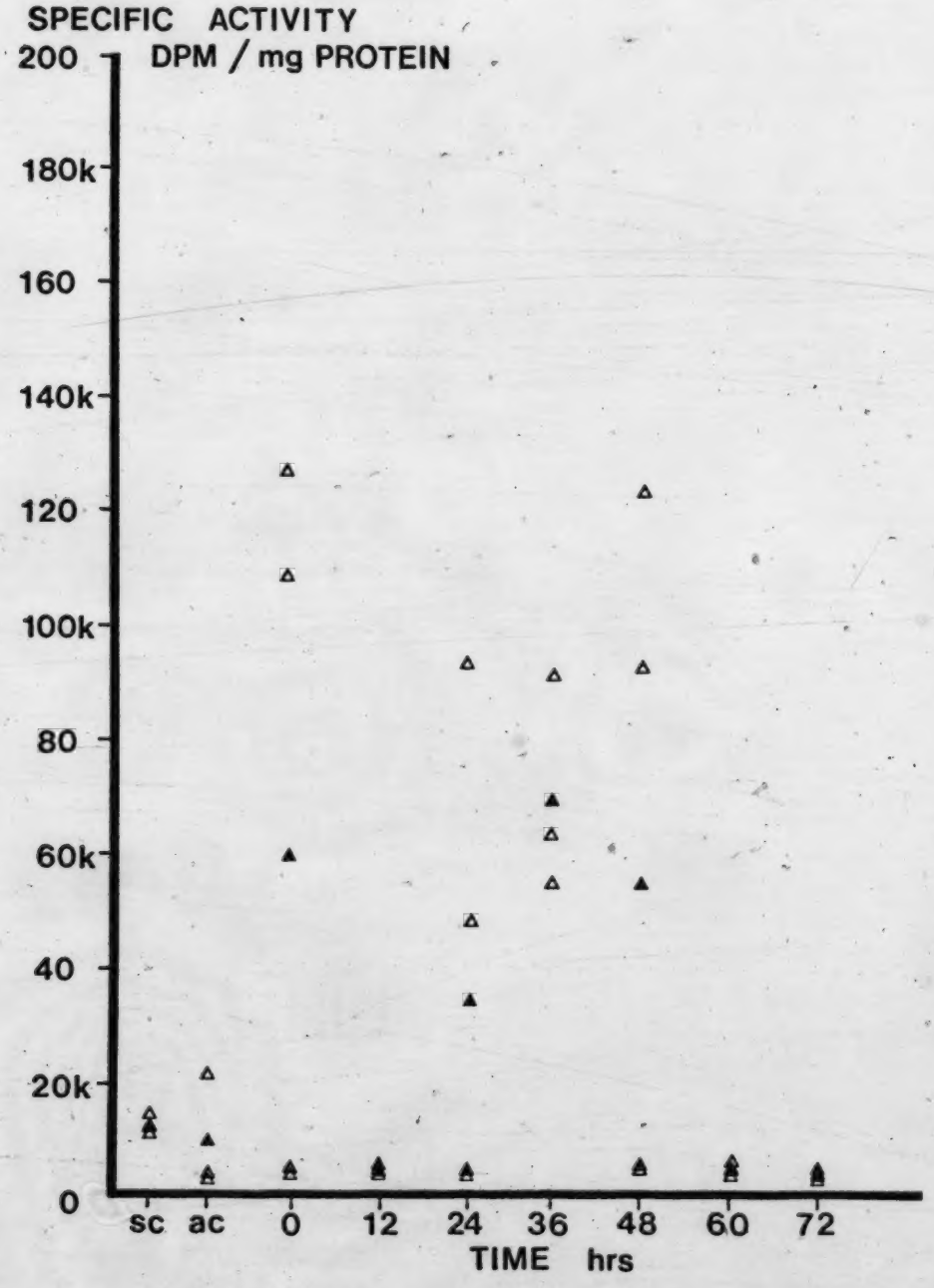

Figure 9. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the total homogenate fraction of soleus muscle from animals of the sprint group (SC-sedentary control; AC-acclimated control) (△-individual animal data; ▲-group mean)

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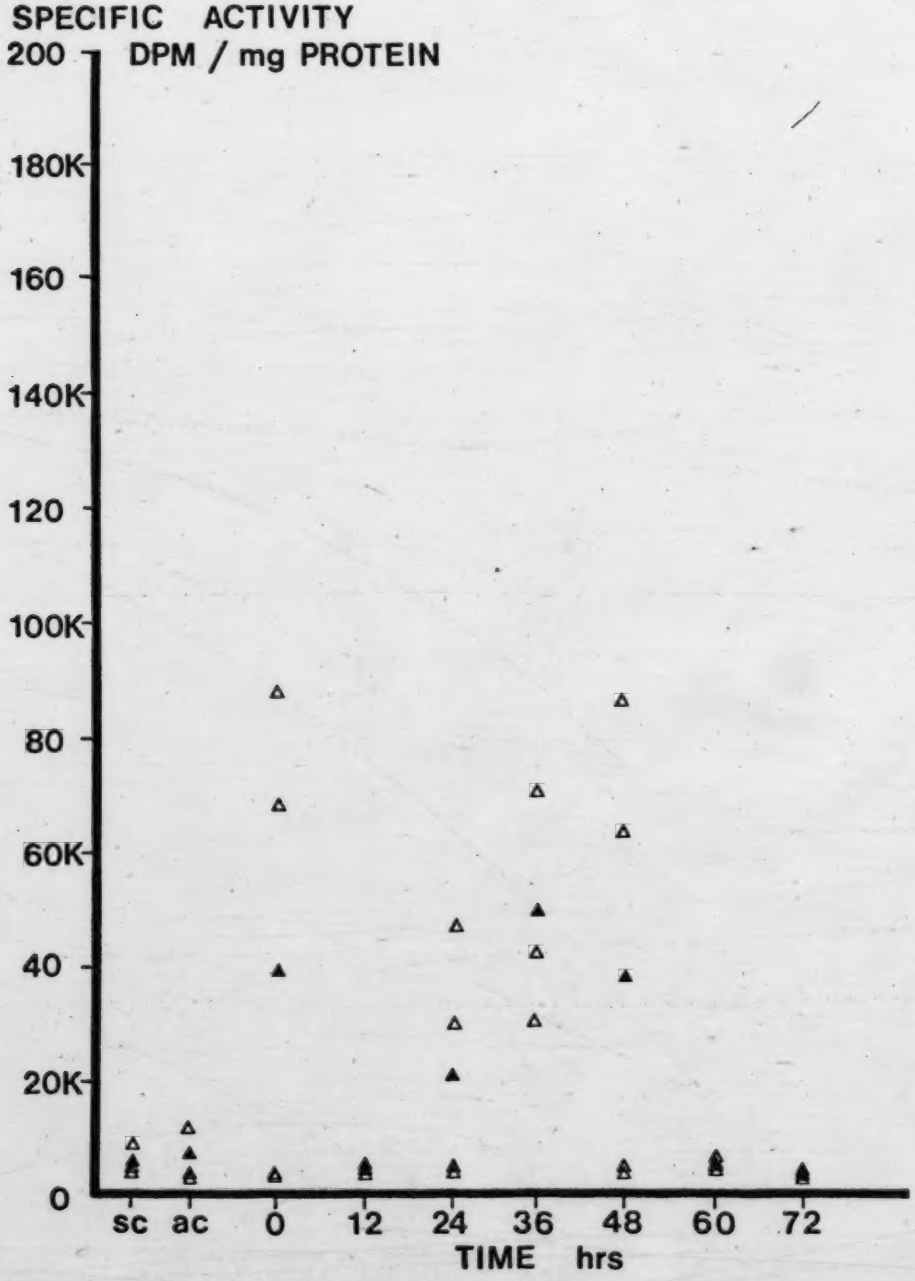

Figure 10. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the myofibrillar-nuclear fraction of soleus muscle from animals of the sprint group (SC-sedentary control; AC-acclimated control) (△-individual animal data;▲-group mean)

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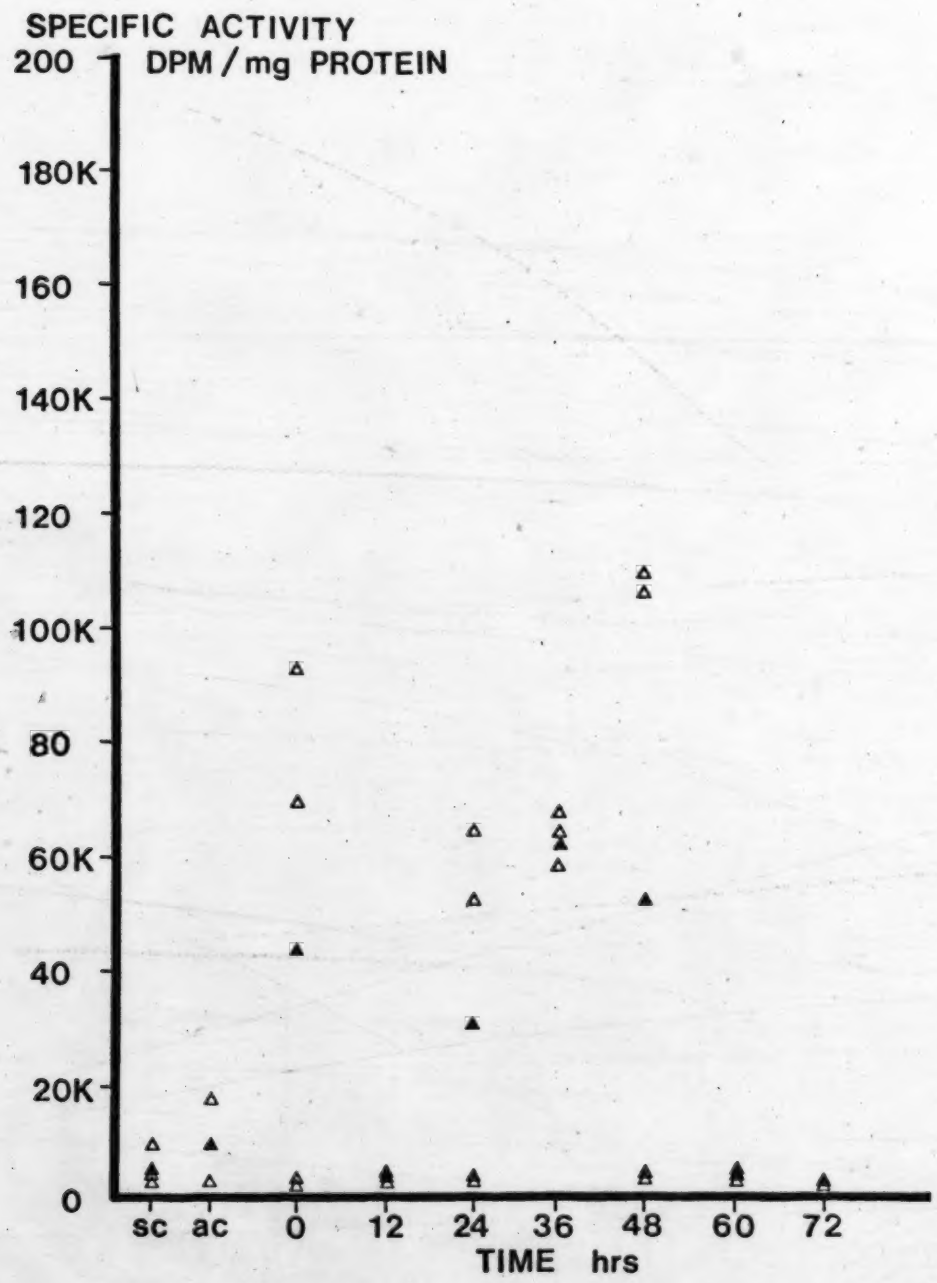

Figure 11. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the mitochondrial fraction of soleus muscle from animals of the sprint group (SC-sedentary control; AC-acclimated control) (△-individual animal data;▲-group mean)

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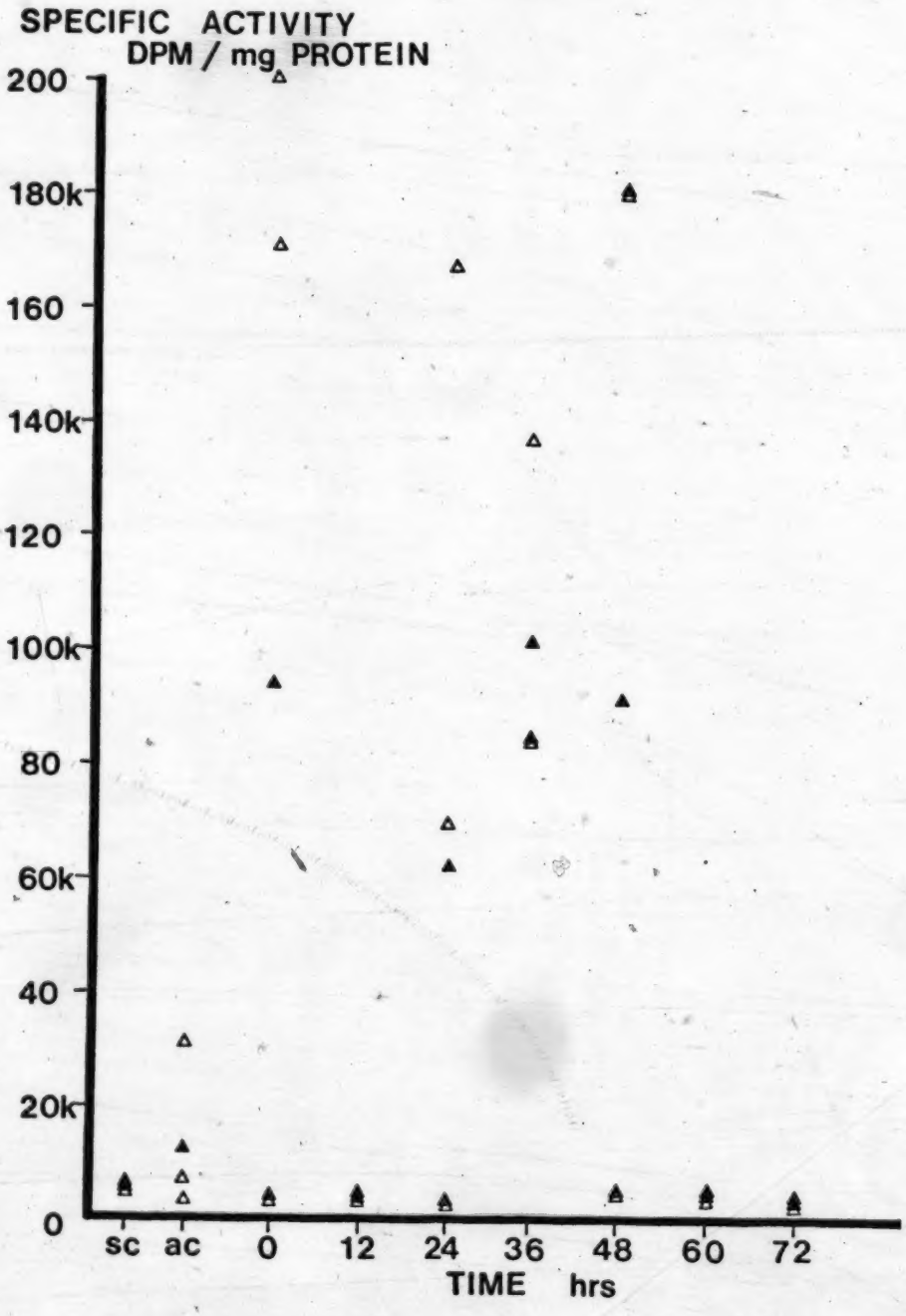

Figure 12. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the soluble fraction of soleus muscle from animals of the sprint group (SC-sedentary control; AC-acclimated control) (△-individual animal data; ▲-group mean)

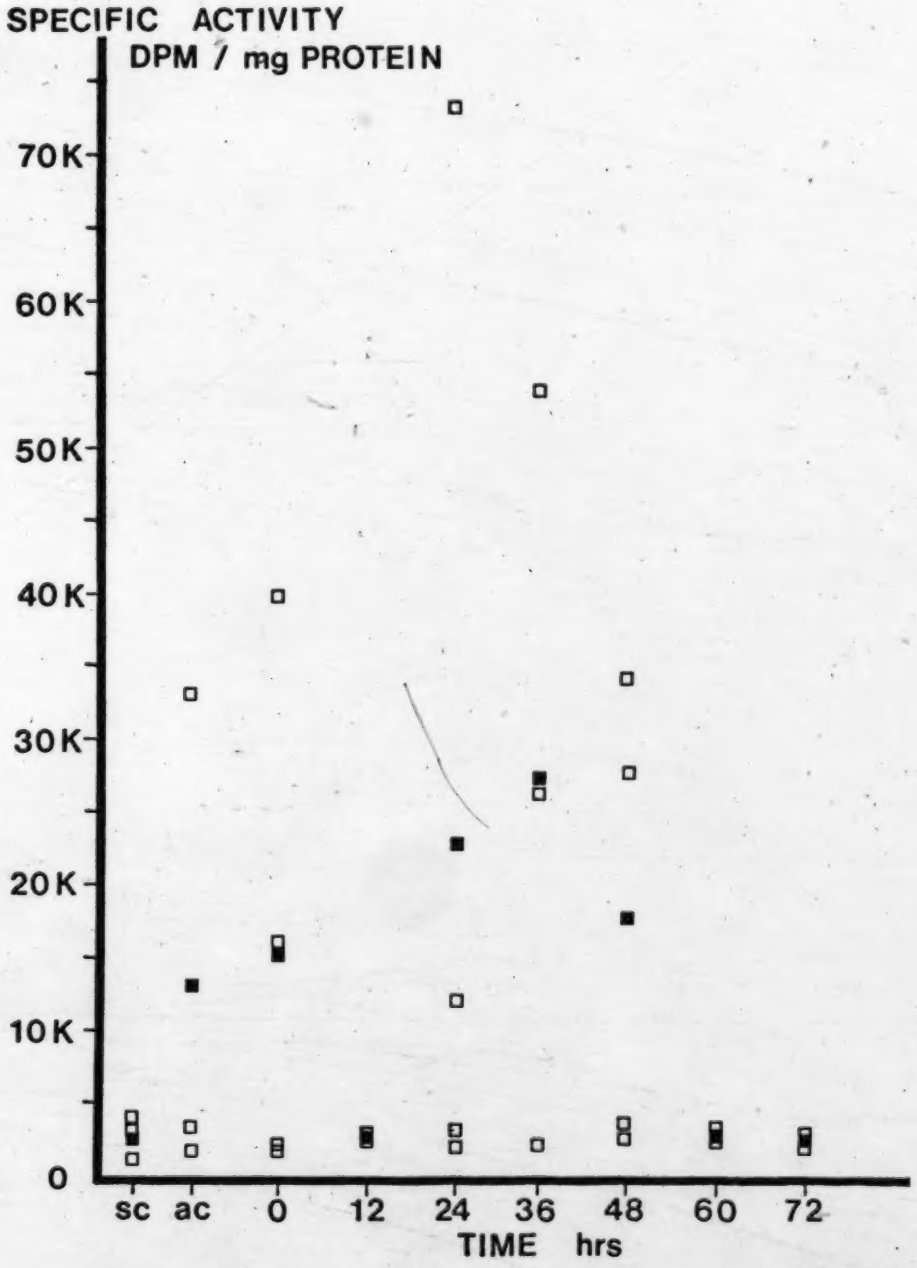

Figure 13. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the total homogenate fraction of plantaris muscle from animals of the sprint group (SC-sedentary control; AC-acclimated control) (□-individual animal data;■-group mean)

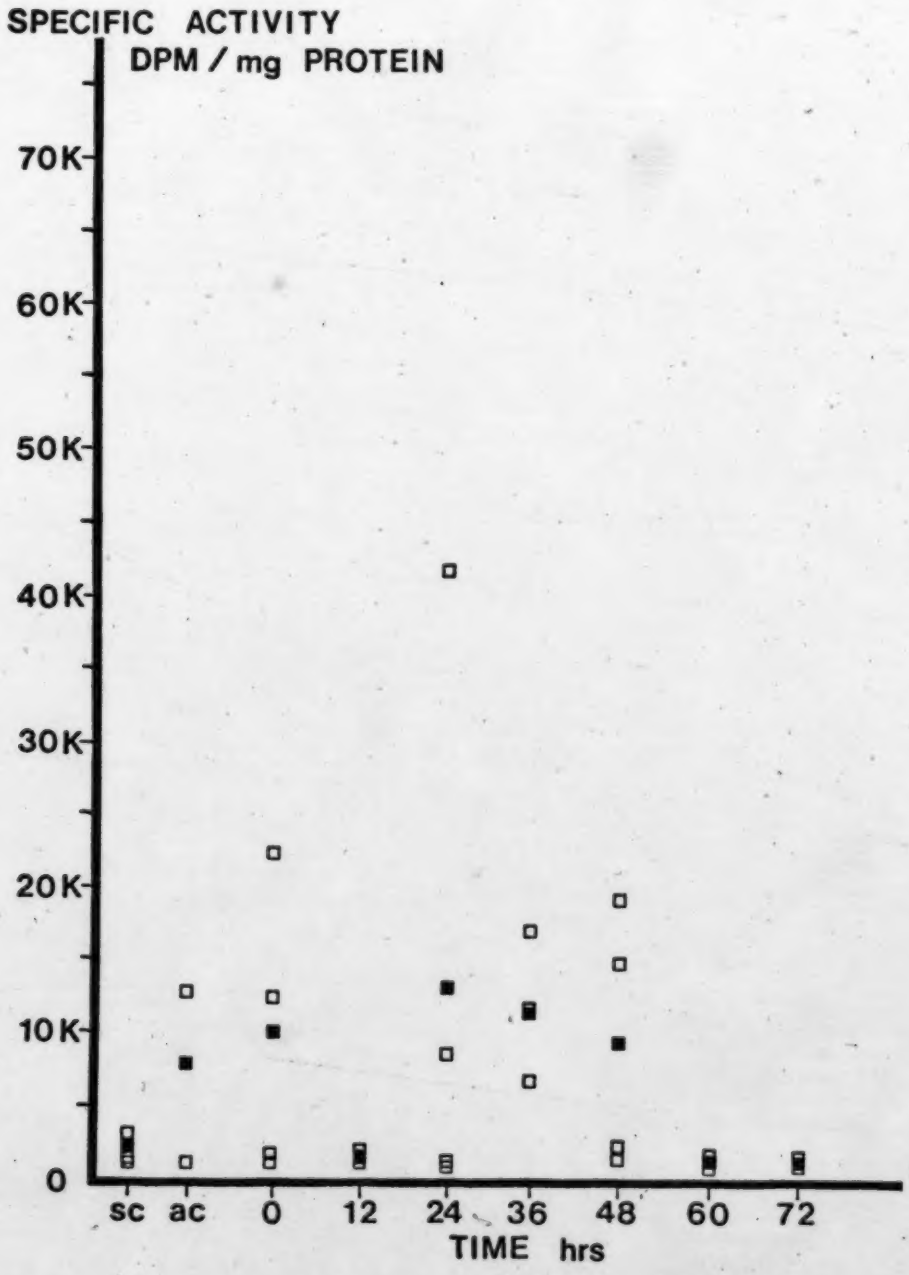

Figure 14. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the myofibrillar-nuclear fraction of plantaris muscle from animals of the sprint group (SC-sedentary control; AC-acclimated control) (□-individual animal data;■-group mean)

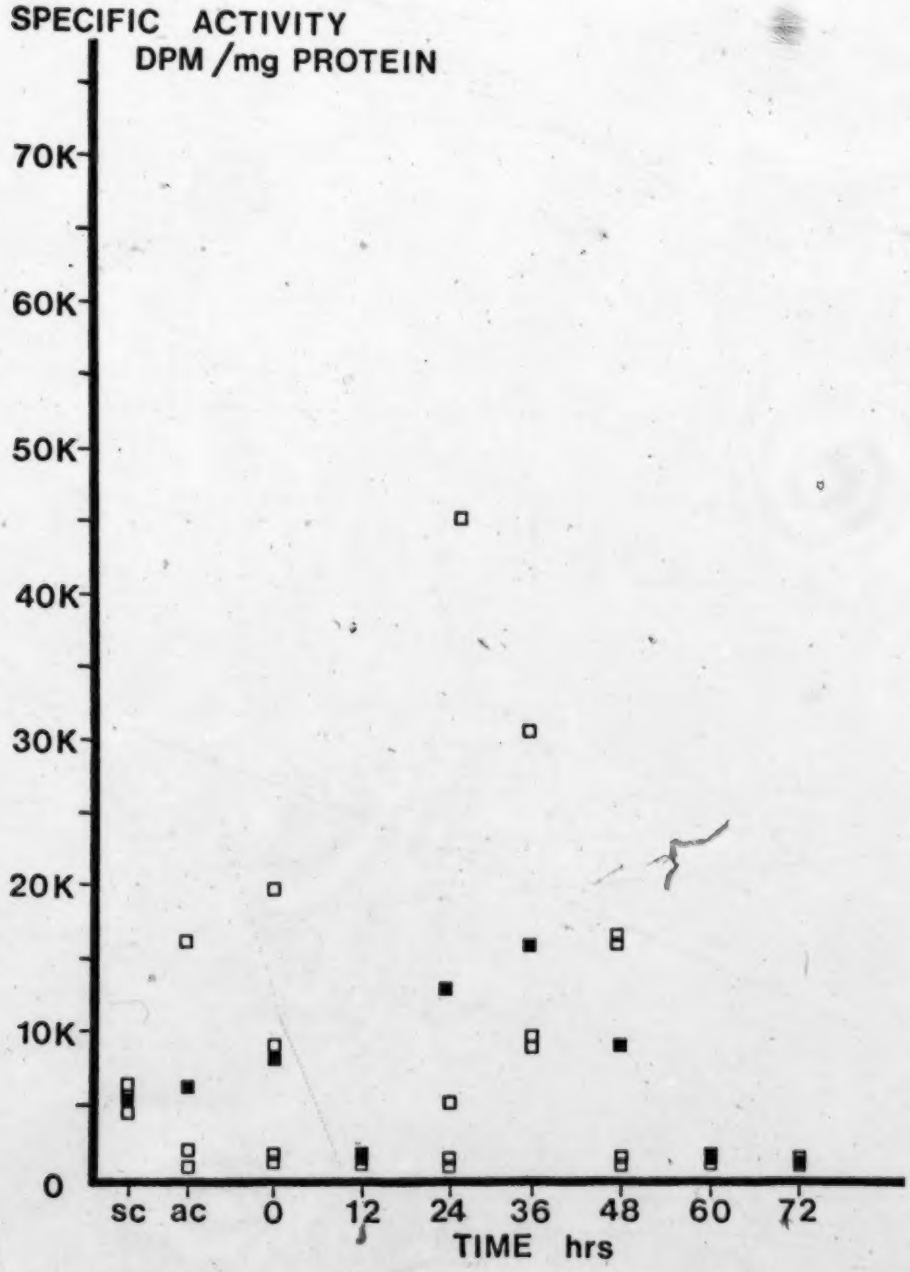

Figure 15. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the mitochondrial fraction of plantaris muscle from animals of the sprint group (SC-sedentary control; AC-acclimated control) (□-individual animal data;■-group mean)

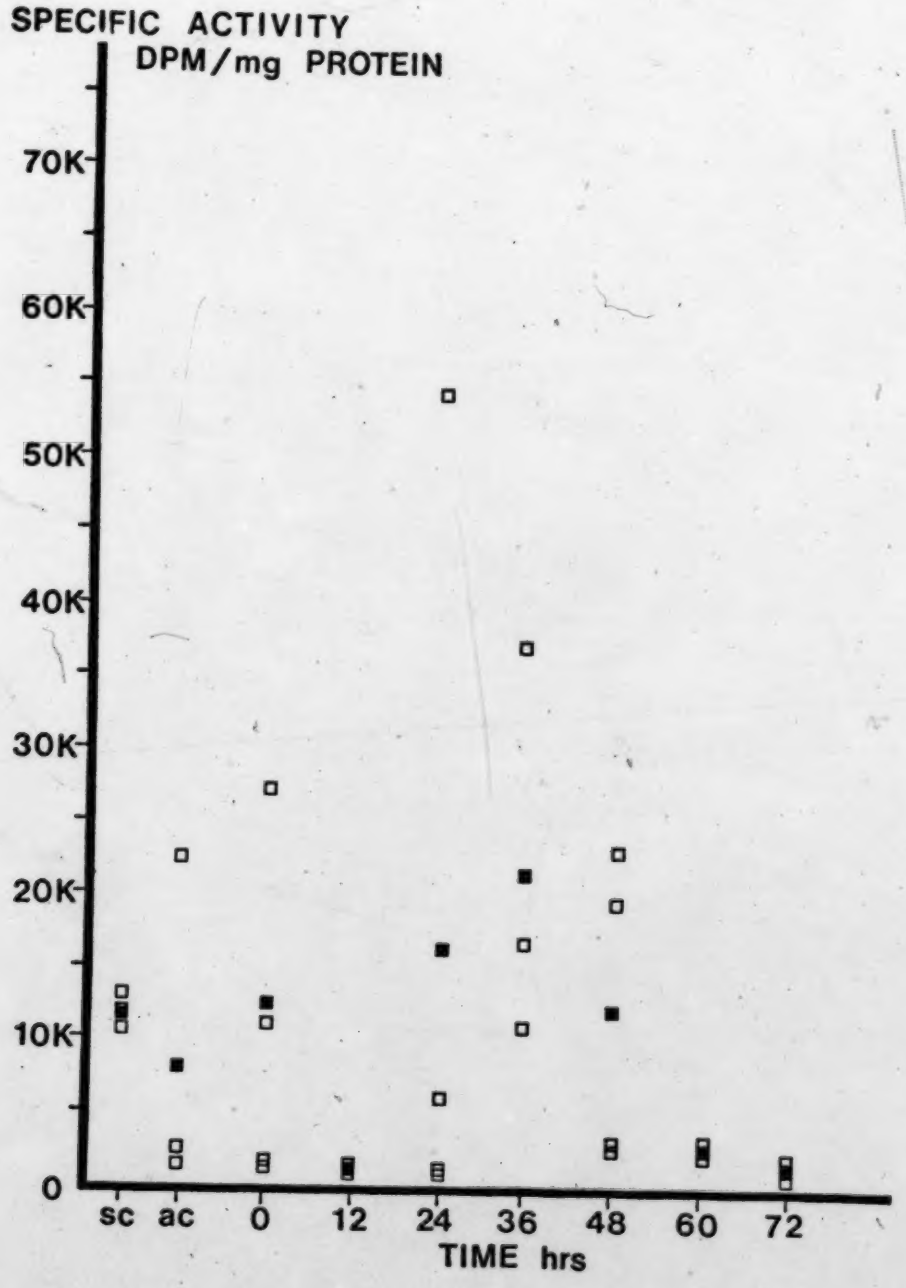

Figure 16. Incorporation of the radionuclide from L-(4,5 ^{3}H)-leucine into protein in the soluble fraction of plantaris muscle from animals of the sprint group (SC-sedentary control; AC-acclimated control) (□-individual animal data;■-group mean)

Table IV. Tritiated leucine incorporation into protein (dpm/mg protein) of all fractions for animals of the aerobic group sacrificed 24 hours post exercise.

Muscle	Animal #	TOT	MYO	MIT	SOL
Soleus	1	174227	118285	92861	279593
	2	84503	77768	86770	123357
	3	3350	2427	2424	1116
Plantaris	1	29398	24399	2231	40756
	2	14239	10775	10117	17385
	3	2636	2074	1320	862
Heart*	1	3781	2566	1993	4559
	2	3685	3009	2258	3490
	3	3123	2223	1620	1911

* - Data used with permission of Mr. D. Wiles.

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Table V. Differences in ^{3}H-leucine incorporation into protein fractions (dpm/mg protein) between animals of the sedentary group (SED.C.) and those of aerobic and anaerobic acclimated control (AE.ACC. and AN.ACC.).

Group	Muscle	Animal #	TOT	MYO	MIT	SOL
SED.C.	S	1	17079	8672	7454	6120
		2	12080	3283	2397	7143
		3	11422	4046	1934	5118
		Mean	13527	5334	3928	6127
	P	1	13152	6936	3499	3350
		2	12313	5707	2106	4107
		3	10318	4710	1646	2965
		Mean	11928	5784	2417	3474
AE.ACC.	S	1	2278	2054	1987	3309
		2	2377	1497		3458
		3	2418	2109	973	3397
		Mean	2358	1887	1480	3388
	P	1	1745	1643	1512	1943
		2	2022	1891		2648
		3	1896	1628	4000	2234
		Mean	1888	1721	2756	2275
AN.ACC.	S	1	20697	12584	19549	34059
		2	2904	1923	1995	4949
		3	1803	1157		2360
		Mean	8468	5221	10772	13789
	P	1	22529	16747	13752	32614
		2	2654	2129	2083	3720
		3	1130	976		1486
		Mean	8771	6617	7918	12607

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Table VI. Distribution (#) of animals showing high and low responses in ^{3}H-leucine incorporation into protein in the response range identified for each of the aerobic and anaerobic groups.

	Muscle	# High response	# Low response	% High * response
Aerobic				
Response Range (12-24 hrs)	S	4	5	44
	P	4	5	44
Anaerobic				
Response Range (24-36 hrs)	S	7	4	64
	P	6	5	55

* - Number of animals which showed elevated ^{3}H-leucine responses expressed as a percentage of the total number of responses in that group. Data represent only those responses seen in the time ranges specified.

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Table VII. Analysis of variance table for blood serum ^{3}H-leucine pools in all animals.

ANALYSIS OF VARIANCE TABLE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARES	F RATIO	PROBABILITY
A*	0.852E+07	1	0.852E+07	3.286	0.076247
B	0.357E+08	8	0.446E+07	1.721	0.118332
AB	0.173E+08	8	0.217E+07	0.809	0.599242
E	0.105E+09	39	0.268E+07		
TOTAL		56			

*A - Treatment Effect

B - Time Effect

AB - Interaction

HOMOGENEITY OF VARIANCE TEST CHISQ= 0.154E+02

PROBABILITY = 0.57045

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Furthermore, the ^{3}H-leucine incorporation pattern observed in either the plantaris or soleus muscles in the same animals followed a similar trend, although the magnitude of response between the two was quite different (Table IV).

The level of ^{3}H-leucine incorporation into protein following acute exercise (figures 1 through 16) suggests that the sprint and endurance exercise differentially affect the ^{3}H-leucine incorporation into muscle proteins. The elevated ^{3}H-leucine uptake after the endurance exercise treatment occurred during the period from 12 to 36 hours post-exercise (figures 1 through 8). On the other hand, following sprint exercise, the response was bimodal with elevations at 0 time and again between 24 and 48 hours (figures 9 through 16), although there are some exceptions (see Table XX).

There was a difference in the amount of ^{3}H-leucine incorporated into protein between SED.C. and exercise acclimated animals (Table V). When the average ^{3}H-leucine incorporation into proteins of total homogenate of muscles from SED.C. animals are compared to those from AE.ACC. animals, there is a six-fold decrease in incorporation of ^{3}H-leucine in acclimated animals. The ^{3}H-leucine incorporation into protein of AN.ACC. animals, when compared to that of SED.C. animals, did not show as great a difference (Table V).

The data also indicate that there is a difference between sprint and endurance groups in the time when

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^{3}H-leucine incorporation returns to control levels. Following endurance exercise, the ^{3}H-leucine incorporation into protein returned to pre-exercise levels in all animals within 48 hours (figures 1 through 8). On the other hand, following sprint exercise, ^{3}H-leucine incorporation returned to pre-exercise levels within 60 hours (figures 9 through 16). The percentage of animals which demonstrated high levels of ^{3}H-leucine incorporation into protein appears to be greater in the sprint group (soleus - 64%, plantaris - 55%) than in the endurance group (soleus - 44%, plantaris - 44%)(Table VI).

The validity of the Analysis of Variance test is dependent upon a number of assumptions, the first of which is related to the homogeneity of variance of the data being tested. As the Chi Square test for Homogeneity of Variance applied to the ^{3}H-leucine incorporation data showed significant heterogeneity of variance, the Analysis of Variance test was judged not suitable and hence will not be presented.

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B. TRANSFER RNA - PROTEIN SYNTHESIS RELATIONSHIP

Since it is known that the first step of *in vivo* protein synthesis is the aminoacylation of transfer RNA, an attempt was made to measure the level of ^{3}H-leucyl-tRNA in soleus and plantaris muscles as a function of time after exercise. Comparison of the skeletal muscle tRNA and leucine incorporation data suggests a reciprocal relationship

between tRNA (S.A. in dpm/OD 260nm) and protein synthesis (represented by the S.A. in dpm/mg protein in the TOT fraction) in skeletal muscle(Tables VIII through XI). When the levels of ^{3}H-leucine bound to tRNA are high, the relative amount of ^{3}H-leucine incorporation into protein is low. This is represented by a high ratio of tRNA:Protein specific activity. On the other hand, when ^{3}H-leucine incorporation into protein is high, the amount of radio-labelled leucine bound to the tRNA molecule is relatively low yielding a low ratio of tRNA:Protein specific activity.

C. METHODOLOGICAL RELIABILITY

The t-test data for protein determinations and radionuclide counting are presented in Tables XII and XIII respectively. T-test values and probabilities reveal that no differences existed between the double aliquots taken for both protein determinations and radionuclide counting.

Analysis of Variance on serum radioactivity data (Table VII) shows that no difference existed between blood ^{3}H-leucine pools in all animals of different experimental groups.

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Table VIII. Relationship between the charging levels of tRNA and protein synthesis in the total homogenate fraction from soleus muscle of animals from the aerobic group.

GROUP	POST-EXER. SACRIFICE	ANIMAL #	tRNA S.A. dpm/OD 260	TOT.HOMOG. S.A. dpm/mg PROTEIN	RATIO tRNA:Pr
SED.C	-	1	454014	17079	26.58
SED.C	-	2	182654	12080	15.12
SED.C	-	3	326501	11422	28.59
AE.ACC.	-	2	322487	2377	135.67
AE.ACC.	-	3	244255	2418	101.02
AE.	0 hr	1	163396	1646	99.27
AE.	0 hr	3	77521	2269	34.17
AE.	24 hr	3	241508	3350	72.09
AE.	48 hr	3	266487	3956	67.36
AE.	60 hr	1	427273	2636	162.09
AE.	60 hr	2	299672	2695	111.20
AE.	60 hr	3	254110	3228	78.72
AE.	72 hr	1	245503	2217	110.74
AE.	72 hr	2	305865	2258	135.46
AE.	72 hr	3	140701	2447	57.50
AE.	12 hr	1	5404	92400	0.0585
AE.	24 hr	1	11471	174297	0.0658
AE.	36 hr	2	113986	95217	1.20

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Table IX. Relationship between the charging levels of tRNA and protein synthesis in the total homogenate fraction from plantaris muscle of animals from the aerobic group.

GROUP	POST-EXER. SACRIFICE	ANIMAL #	tRNA S.A. dpm/OD 260	TOT.HOMOG. S.A. dpm/mg PROTEIN	RATIO tRNA:Pr
SED.C.	-	1	140450	13152	10.68
SED.C	-	2	40264	12313	3.27
SED.C	-	3	86560	10318	8.39
AE.ACC.	-	2	254474	2022	125.85
AE.ACC.	-	3	96971	1896	51.15
AE.	0 hr	1	140500	1847	76.07
AE.	0 hr	3	60191	1727	34.85
AE.	24 hr	3	161587	2636	61.30
AE.	36 hr	2	39888	10032	3.98
AE.	48 hr	3	33060	2965	11.15
AE.	60 hr	1	97014	1809	53.63
AE.	60 hr	2	55198	2068	26.69
AE.	60 hr	3	59824	2488	24.05
AE.	72 hr	1	51545	2229	23.12
AE.	72 hr	2	224892	1585	141.89
AE.	72 hr	3	64141	1789	35.85
AE.	12 hr	1	12171	27848	0.4371
AE.	24 hr	1	9668	29398	0.3289

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Table X. Relationship between the charging levels of tRNA and protein synthesis in the total homogenate fraction from soleus muscle of animals from the anaerobic group.

GROUP	POST-EXER. SACRIFICE	ANIMAL #	tRNA S.A. dpm/OD 260	TOT.HOMOG. S.A. dpm/mg PROTEIN	RATIO tRNA:Pr
SED.C	-	1	454014	17079	26.58
SED.C	-	2	182654	12080	15.12
SED.C	-	3	326501	11422	28.59
AN.	0 hr	1	66341	2307	28.76
AN.	0 hr	2	158467	2042	77.60
AN.	12 hr	1	323011	2534	127.47
AN.	12 hr	2	105794	3134	33.76
AN.	12 hr	3	387100	2660	145.53
AN.	24 hr	1	73635	47342	1.56
AN.	24 hr	2	293167	1803	162.60
AN.	24 hr	3	474475	2243	211.54
AN.	48 hr	1	16537	3298	5.01
AN.	48 hr	2	244299	3784	64.56
AN.	48 hr	4	423253	122530	3.45
AN.	60 hr	1	348427	3414	102.06
AN.	60 hr	2	422554	2575	164.10
AN.	60 hr	3	120709	3233	37.34
AN.	72 hr	1	325286	3120	104.26
AN.	72 hr	2	331537	2543	130.37
AN.	72 hr	3	287528	664	433.02

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Table X (cont'd)... Relationship between the charging levels of tRNA and protein synthesis in the total homogenate fraction * from soleus muscle of animals from the anaerobic group.

GROUP	POST-EXER. SACRIFICE	ANIMAL #	tRNA S.A. dpm/OD 260	TOT.HOMOG. S.A. dpm/mg PROTEIN	RATIO tRNA:Pr
AN.ACC.	-	1	15555	20697	0.7516
AN.	0 hr	3	16630	124950	0.1331
AN.	0 hr	4	18404	109404	0.1682
AN.	24 hr	4	17370	95051	0.1827
AN.	36 hr	1	16228	92505	0.1754
AN.	36 hr	2	35373	54799	0.6455
AN.	36 hr	3	15722	61922	0.2539
AN.	48 hr	3	24166	93589	0.2582

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Table XI. Relationship between the charging levels of tRNA and protein synthesis in the total homogenate fraction from plantaris muscle of animals from the anaerobic group.

GROUP	POST-EXER. SACRIFICE	ANIMAL #	tRNA S.A. dpm/OD 260	TOT.HOMOG. S.A. dpm/mg PROTEIN	RATIO tRNA:Pr
SED.C.	-	1	140450	13152	10.68
SED.C.	-	2	40264	12313	3.27
SED.C.	-	3	86560	10318	8.39
AN.	0 hr	1	19392	1829	10.60
AN.	0 hr	2	46663	1451	32.16
AN.	12 hr	1	216835	2089	103.80
AN.	12 hr	2	43686	2112	20.69
AN.	12 hr	3	96330	2010	47.83
AN.	24 hr	1	49291	6709	7.35
AN.	24 hr	2	143454	1497	95.83
AN.	24 hr	3	16343	1911	113.21
AN.	48 hr	1	13004	3009	4.32
AN.	48 hr	2	85494	3525	24.25
AN.	48 hr	4	157072	19284	8.15
AN.	60 hr	1	255697	2403	106.41
AN.	60 hr	2	114321	2217	51.57
AN.	60 hr	3	270970	2741	98.86
AN.	72 hr	1	116989	2502	46.76
AN.	72 hr	2	183510	1934	94.89
AN.	72 hr	3	140424	906	154.99

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Table XI (cont'd)... Relationship between the charging levels of tRNA and protein synthesis in the total homogenate fraction from plantaris muscle of animals from the anaerobic group.

GROUP	POST-EXER. SACRIFICE	ANIMAL #	tRNA S.A. dpm/OD 260	TOT.HOMOG. S.A. dpm/mg PROTEIN	RATIO tRNA:Pr
AN.ACC.	-	1	12590	22529	0.558
AN.	0 hr	3	14291	27184	0.5257
AN.	0 hr	4	14807	11195	1.32
AN.	24 hr	4	16910	54004	0.3131
AN.	36 hr	1	11591	10123	1.15
AN.	36 hr	2	21652	16365	1.32
AN.	36 hr	3	13272	37190	0.3569
AN.	48 hr	3	21501	22780	0.9439

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Table XII. Data used to determine t-test relationship between double aliquots of protein samples taken from soleus (S) and plantaris (P) muscles.

GROUP	ANIMAL#	MUSCLE	FRACTION	ALIQUOT 1 OD260	ALIQUOT 2 OD260
SED.C.	2	S	TOT	.206	.210
			MYO	.665	.573
			MIT	.232	.227
			SOL	.175	.175
		P	TOT	.210	.208
			MYO	.377	.373
			MIT	.173	.176
			SOL	.236	.229
AE.ACC.	1	S	TOT	.189	.192
			MYO	.290	.288
			MIT	.119	.117
			SOL	.155	.156
		P	TOT	.213	.212
			MYO	.327	.323
			MIT	.140	.143
			SOL	.202	.202
AN. 0	3	S	TOT	.172	.173
			MYO	.300	.303
			MIT	.114	.112
			SOL	.154	.158
		P	TOT	.130	.131
			MYO	.351	.354
			MIT	.144	.142
			SOL	.266	.266
AE. 24	1	S	TOT	.179	.183
			MYO	.355	.379
			MIT	.241	.236
			SOL	.154	.152
		P	TOT	.214	.211
			MYO	.357	.359
			MIT	.173	.167
			SOL	.209	.211

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Table XII (cont'd)... Data used to determine t-test relationship between double aliquots of protein samples taken from soleus (S) and plantaris (P) muscles.

GROUP	ANIMAL#	MUSCLE	FRACTION	ALIQUOT 1 OD260	ALIQUOT 2 OD260
AN. 72	2	S	TOT	.179	.181
			MYO	.396	.380
			MIT	.253	.245
			SOL	.174	.178
		P	TOT	.206	.208
			MYO	.317	.330
			MIT	.166	.167
			SOL	.245	.247
			Means =	.234	.232

T test Values for Means

Column	1	2
Row 1	0.0	-0.047
Row 2	0.047	0.0

Degrees of Freedom = 39

Probabilities of T for Differences between Means

Column	1	2
Row 1	1.000	0.963
Row 2	0.963	1.000

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Table XIII. Data used to determine t-test relationship between double aliquots of radioactivity samples taken from soleus (S) and plantaris (P) muscles.

GROUP	ANIMAL#	MUSCLE	FRACTION	ALIQUOT 1 cpm	ALIQUOT 2 cpm
SED.C.	2	S	TOT	2614	3326
			MYO	3811	3765
			MIT	222	308
			SOL	378	499
		P	TOT	2966	3142
			MYO	3502	3514
			MIT	157	146
			SOL	397	482
AE.ACC.	1	S	TOT	608	601
			MYO	1015	1023
			MIT	91	93
			SOL	220	228
		P	TOT	552	571
			MYO	927	997
			MIT	105	110
			SOL	211	225
AN. 0	3	S	TOT	28338	29314
			MYO	48980	46300
			MIT	2718	2687
			SOL	12763	12725
		P	TOT	3491	3495
			MYO	13653	13113
			MIT	1286	1255
			SOL	5950	6053
AE. 24	1	S	TOT	39733	41540
			MYO	82500	80600
			MIT	11450	11876
			SOL	15186	15531
		P	TOT	9364	9043
			MYO	15838	16808
			MIT	1610	1551
			SOL	4268	4092

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Table XIII (cont'd)... Data used to determine t-test relationship between double aliquots of radioactivity samples taken from soleus (S) and plantaris (P) muscles.

GROUP	ANIMAL#	MUSCLE	FRACTION	ALIQUOT 1 cpm	ALIQUOT 2 cpm
AN. 72	2	S	TOT	602	620
			MYO	823	874
			MIT	140	150
			SOL	275	286
		P	TOT	606	593
			MYO	814	831
			MIT	134	139
			SOL	347	336
			Mean =	7966	7955

T Test Values for Means

Column	1	2
Row 1	0.0	0.812
Row 2	-0.812	0.0

Degrees of Freedom = 39

Probabilities of T for Differences between Means

Column	1	2
Row 1	1.000	0.422
Row 2	0.422	1.000

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IV. DISCUSSION

The discussion which follows will be presented in four sections: 1) Methodological Reliability, 2) Tritiated Leucine Incorporation into Protein, 3) Transfer RNA - Protein Synthesis Relationship, and 4) Practical Implications.

A. METHODOLOGICAL RELIABILITY

The ^{3}H-leucine incorporation and ^{3}H-leucyl-tRNA data presented in figures 1 through 16 and Tables VIII through XI, respectively, suggest differences in the responses of soleus and plantaris muscles to sprint or endurance work. These differences are statistically shown by the significant Chi Square (for Homogeneity of Variance) obtained from ^{3}H-leucine incorporation data.

It is important to describe at the outset of the discussion the steps taken to ensure that the observed differences in data were not due to errors in methodological technique, but to the variable biological response of each animal to the imposed exercise load.

The comparison by t-test between the indepently processed duplicate samples taken from each fraction of both muscles showed no difference (Tables XII and XIII). This would indicate that these analytical techniques were reliable and were not the cause of the changes in the radioactive labelling of proteins and tRNA.

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Also, the Analysis of Variance of the serum radioactivity data (Table VII) showed no difference in serum tritium activity between the different experimental groups. This would suggest that the ^{3}H-leucine pool was constant between groups and variations in precursor pool were not responsible for the differences seen in the protein labelling data.

As well, the sacrifice schedule (Table XXII, Appendix D) shows that the time of sacrifice for each animal within the same group occurred at different times of day, *ie.* rats were not sacrificed at any particular time of their daily cycle. There was no relationship between the time of day (or night) when an animal was sacrificed and the ^{3}H-leucine incorporation into different fractions of soleus and plantaris muscle. Thus, the possibility that diurnal variation was the cause of the variability in ^{3}H-leucine incorporation into protein is remote.

Lastly, the general pattern in the observed changes occurred in a specific time range following the different exercise treatments (*cf.* AE. - 12 to 36 hrs; AN. - at 0 time and from 24 to 48 hrs). Since the sacrifice was random, it is unlikely that the order of sacrifice was the cause of the measured differences in the data.

As discussed above, the observations that the ^{3}H-leucine incorporation is increased during a specific period of time supports the hypothesis that the demonstrated differences in ^{3}H-leucine incorporation into protein was not

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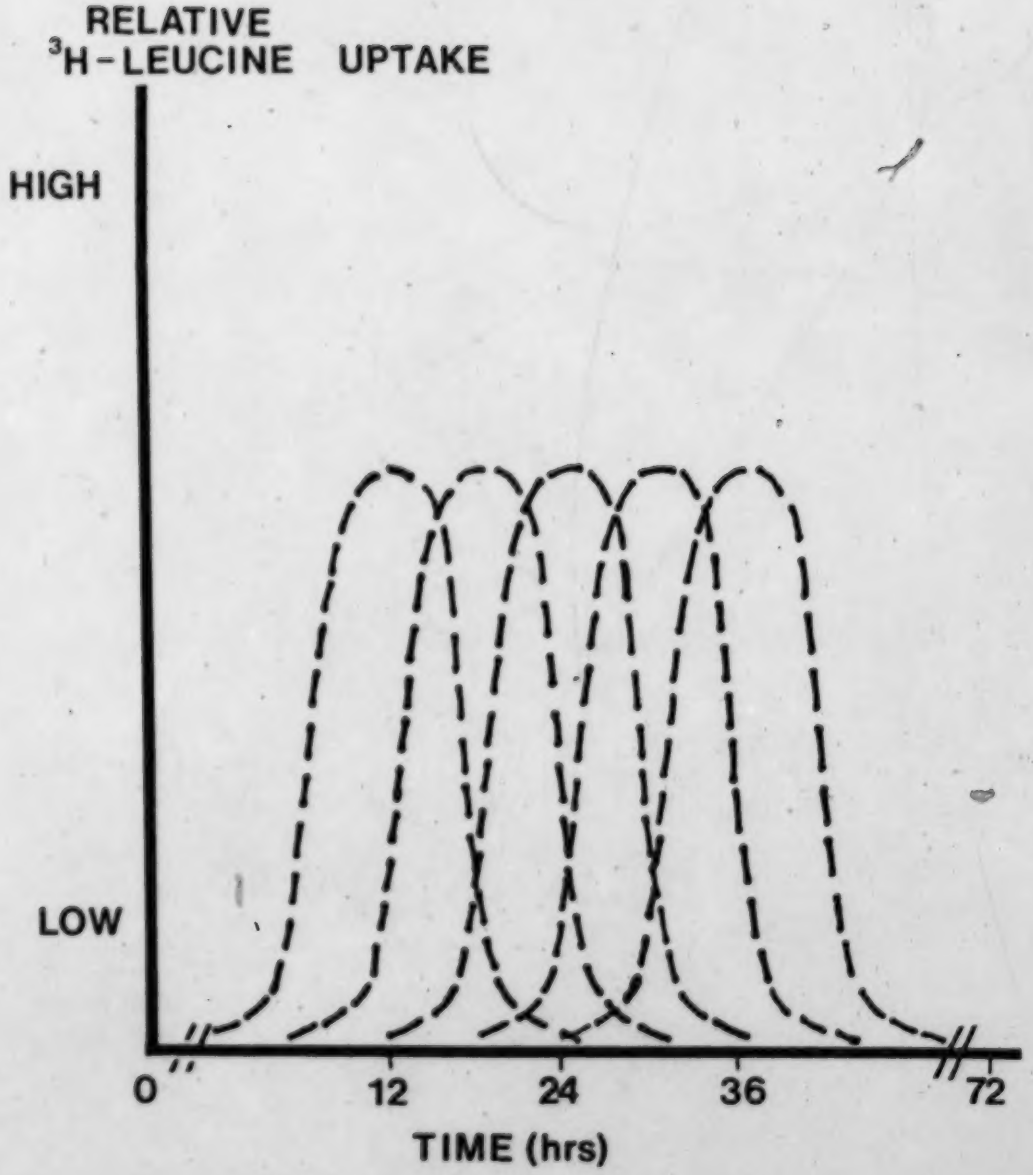

Figure 17. Hypothesized distribution pattern of endurance acclimated animals showing peaks in ^{3}H-leucine incorporation into protein. (--- individual animal pattern)

a result of methodological error, and that individual biological differences among animals must, in some way, account for the pattern of responses seen as a result of the two types of acute exercise. Although the physical intensity of the exercise bouts imposed on these animals can be calculated, the *relative physiological stress* of such work on each animal is difficult to quantify. Even if the work is of equal intensity, a difference in relative stress may effect responses which vary both qualitatively and/or quantitatively in any system within different animals. If one assumes that a stress of significant magnitude imposed on a certain muscle causes some altered biochemical state - in particular pertaining to protein metabolism - then each individual animal would reflect such an altered state in terms of accelerated protein turnover (as observed in ^{3}H-leucine incorporation). However, because of the physiological individuality evident in each animal, how rapidly the sign of stress appears in muscle may not be synchronous in all animals. Though it might be an over simplification, each animal may respond to the stress as depicted by the broken lines in Fig. 17.

Other alternatives which might explain the measured differences in response patterns between animals include: 1) the consideration that the work done on the performance days was stressful for some animals and not for others. Hence, only those animals which were stressed showed elevated ^{3}H-leucine responses, 2) the consideration that the work

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performed was stressful enough in some animals to suppress ^{3}H-leucine uptake during the times chosen for observation, and 3) the possibility that in those animals which showed no elevated responses in ^{3}H-leucine uptake, changes in protein turnover were accomplished by decreasing the degradation of protein and not by altering synthesis.

If, as hypothesized, individual animal response patterns in ^{3}H-leucine incorporation do occur within such a range of post-exercise sacrifice times (*cf*. AE. - 12 to 36 hrs; AN. - bimodally with elevations at 0 time and again between 24 and 48 hrs), the sample times assigned to each animal may not represent elevated response times for different animals within those groups. Thus, if an animal whose individual response pattern shows a peak at 36 hours was randomly assigned to the 12 hour sacrifice group, the measured response in that animal at the 12 hour sacrifice time could be decreased. It is possible that animals which showed an elevated ^{3}H-leucine response at a specific post-exercise time where change was seen, represent those animals which show an elevated response time which coincides with the sacrifice time (eg. Animal 24 #1,2 soleus muscle - Table XIX). On the other hand, animals which show low levels of ^{3}H-leucine incorporation into protein at the same sacrifice time (eg. Animal 24 #3 soleus muscle - Table XIX) could represent those animals which demonstrate a peak in ^{3}H-leucine incorporation into protein which could have occurred either prior to or following the sacrifice time in

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question. If we examine the group means calculated for the ^{3}H-leucine incorporation data an elevated mean is seen at 24 hours following endurance work (Table XIX, appendix C) and 36 hours following sprint work (Table XX, Appendix C).

It is of interest to note that following spint exercise the elevated response occurred prior to (*ie.* 0 time) and again at 36 hours when compared to the endurance exercise. Though no specific reason for this can be offered, it is possible that the intermittant high-intensity nature of the exercise elicits the bimodal response.

Because of the dearth of information available on protein synthesis following acute exercise and the methodological differences that exist between the few which are published and the present investigation, a comparison with related investigations is difficult.

In the present study, rats, after having performed a treadmill running acclimation program, were required to perform an experimental exercise bout (either sprint or endurance) on two consecutive days. Soleus and plantaris muscles were sampled prior to exercise, and at 12 hour intervals from 0 to 72 hours following the last exercise session. Ten minutes prior to sacrifice, L-(4,5 ^{3}H)-leucine was administered by intravenous injection at a dosage of 25μCi/100g body weight. *In vivo* incorporation of ^{3}H-leucine into proteins and the level of aminoacylation of leucine onto tRNA were measured.

In contrast, Dohm and co-workers (1977a, 1977b, 1978)

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studied protein metabolism in response to chronic exercise in which rats were trained from 6 to 12 weeks on an endurance type of treadmill running program. The gastrocnemius muscle was examined and ^{14}C-leucine was the radio-labelled amino acid used. They studied *in vivo* and *in vitro* models, administering ^{14}C-leucine at maximum dosages of 15μCi/100g and 0.2μCi/100g, respectively. The animals were sacrificed from 1 to 3 hours post-injection. In their report, no information was given regarding the time elapsed since the last exercise session. Levels of ^{14}C-leucine incorporation into protein fractions in these studies are lower by one order of magnitude (349 ± 22 dpm/mg protein in total homogenate fraction of soleus muscle from an untrained animal), when compared to the levels measured in the present investigation. It is possible that the type and duration of work performed, the tissue preparation, the dose and method of label administration, the duration of *in vivo* incorporation, and the time elapsed since the last exercise session could account for the differences in magnitude observed in both situations.

Beecher *et al* (1979) also used chronically trained male rats. The animals were sacrificed 1 hour following intravenous administration of (alpha - ^{14}C)-aminoisobutyric acid (1.0μCi/100g). Gastrocnemius muscle was chosen and the authors gave no information regarding the elapsed time after the last exercise session.

Rogers and colleagues (1979), on the other hand, used

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mature untrained guinea pigs to study protein synthesis following a single run to exhaustion on a motor driven treadmill at a slow speed and a low intensity characteristic of endurance work. The animals were sacrificed 1 hour following the exhaustive run and tissue slices from gastrocnemius and soleus muscles were incubated in a medium containing 0.05mCi of ^{3}H-leucine for a period of between 0.5 and 1.5 hours. The specific activities of proteins, RNA, polysomes, microsomes, and the total homogenate fraction of tissue slices were measured. A representative value of the level of ^{3}H-leucine in the total homogenate fraction of control soleus tissues from the study conducted by Rogers *et al.* is 16,300 ± 650 cpm. This ^{3}H-leucine result would appear to differ significantly from those of the present investigation (2000-3000 dpm/mg protein). The type of animal, the duration of the training sessions, the exhaustive nature of the work bouts, the *in vitro* method of ^{3}H-leucine incubation, the tissue preparation, and the time of sacrifice post exercise could all contribute to these observed differences.

McManus and co-workers (1975) also used guinea pigs trained on an endurance program over a period of 5 weeks. The experimental design included castration, testosterone replacement, and normal gonadal function treatments. The animals were sacrificed 18 hours following their last run on the treadmill. Four hours prior to sacrifice, all animals received, by intravenous injection, L-(4,5 ^{3}H)-leucine at a

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dosage of 5uCi/100g. Protein content of plantaris muscle was determined and labelled amino acid incorporation was expressed as cpm/mg wet muscle weight. The ten-fold higher results of the present investigation as compared to that of McManus *et al* (21.0 ± 2.0 cpm/mg tissue) could be attributed to the differences between the animals used, the length of the training programs, the method of label administration, the duration of label incubation, the dosage of label used, and/or the time elapsed since the last exercise session.

Finally, Wenger *et al* (in press) reported the results of our previous study in which rats were acclimated to one of either sprint or endurance protocols on a motor driven rodent treadmill. Animals were sacrificed before exercise and at 0, 2, 18, 24, or 48 hours post-exercise. Slices of red and white vastus lateralis muscles were incubated in a medium containing 10μCi/ml of L-(4,5 ^{3}H)-leucine, and the incorporation of the radionuclide was measured (dpm/mg protein) in the whole homogenate and in four subcellular fractions. The experimental work bout was performed on only 1 day and was equal in distance to the two-day protocol outlined in the present study. The results observed in our earlier study range from one to two orders of magnitude less than those measured during the present investigation. The different results seen between these two studies would likely be due to the different performance protocols used, the *in vivo* vs *in vitro* incubation techniques or some combination of these two factors.

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In summary, in studying the effects of exercise on muscle protein metabolism, there are a number of experimental variables such as:

- sampling time relative to the last exercise session (Bostrom *et al*, 1974; present study);
- the duration of *in vivo* incorporation of the radio-labelled amino acid prior to sacrifice (Martin *et al*, 1977);
- species of experimental animals (guinea pigs, rats)(Burleigh, 1974);
- muscle tissues (gastrocnemius, soleus, plantaris, red and white vastus lateralis);
- incubation media and methods of *in vivo* and *in vitro* incorporation (Zak *et al.*, 1979); and
- the type, intensity of exercise, and total amount of work imposed on the experimental animals

which could contribute to the differences in the results reported in the various investigations.

B. TRITIATED LEUCINE INCORPORATION INTO PROTEIN

The data from both soleus and plantaris muscles from all animals (figures 1 through 16) indicate that changes in the incorporation of ^{3}H-leucine into protein in the four fractions of skeletal muscle (TOT, MYO, MIT, and SOL) occurred at the same time and in the same direction (Table IV). This would suggest that the different fractions of skeletal muscle do not respond independently of one another,

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but are modified in concert. In this study, the ^{3}H-leucine incorporation into protein was terminated within 15 minutes. During the first 10 to 15 min. after ^{3}H-leucine incorporation, the amount of labelled proteins degraded is less than 10-15 / 300 of the total labelled proteins (if the half-life of protein is assumed to be 5 hours). In fact, half-lives of most proteins are in the order of days. (Note: there are some proteins such as membrane components and neurotransmitter receptors that turnover in minutes, but they constitute a very small proportion of the fractions studied.) Thus, the incorporation measured in this study, can be regarded as an indication of the rate of protein synthesis (Zak *et al.*, 1979). The elevated protein synthetic activity appears to be a general response in both skeletal muscles examined (although the heart muscle does not show a similar pattern (Table IV)). However, specific protein fractions have been shown to differ in metabolic responses as a result of various acute exercise stimuli (Wenger *et al*, in press; McManus *et al*, 1975) or chronic training regimens (Dohm *et al*, 1977a, 1978; Holloszy, 1975; Baldwin *et al*, 1977; Sjodin *et al*, 1976; Goldberg, 1975; Jaweed *et al*, 1974; Gordon, 1967). Discrepancy between the results of this and other studies cannot be explained by available information, but lack of statistical significance may be directly related to the small number of animals per group.

The six-fold decrease in the incorporation of ^{3}H-leucine into the total homogenate fraction of muscles

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from AE.ACC. animals (soleus - 2358 dpm/mg protein; plantaris - 1888) when compared to the SED.C. animals (soleus - 13527; plantaris - 11928), indicates that the aerobic acclimation modifies the incorporation rate of ^{3}H-leucine into protein, and hence, the protein synthetic activity in skeletal muscle. When the average ^{3}H-leucine incorporation into protein of SED.C. animals is compared to those of AN.ACC. animals (soleus - 8468; plantaris - 8771), the difference does not appear to be as great (Table V). If, however, the individual values of ^{3}H-leucine incorporation of the AN.ACC. group presented in Table V are considered, it becomes apparent that the response of the number 1 animal in that group (ie. AN.ACC. #1) substantially inflates the group mean. The reason for the unusually elevated ^{3}H-leucine incorporation in this animal is not clear but will be discussed in a later section. If the individual data for the remaining two animals of that group are considered, independent of the elevated response of the AN.ACC. 1 animal, the range of values in ^{3}H-leucine incorporation into protein is similar to that shown in animals of the AE.ACC. group. Thus, it would appear that, in general, exercise acclimation protocols such as those presented in this study, whether they are of sprint or endurance nature, are capable of depressing the rate of protein synthetic activity in skeletal muscle. Alterations in protein turnover rates suggested from evidence of increased protein catabolism as a result of endurance training presented by Dohm *et al*

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(1977a), as well as evidence of increased synthesis of certain enzymes (Cytochrome C - Booth and Holloszy (1975)) and increased RNAase activity following endurance training (Szczesna-Kazzmarek *et al* (1978)), would tend to support these findings (at least with respect to the AE.ACC. group).

The consistent response times range from 12 to 36 hours for the endurance group and the dual responses at 0 hours and 24 to 48 hours for the sprint group. As the distance covered by both groups was equal, either the interval vs. continuous nature of the work performed, the intensity of the exercise, or some combination of these two factors would seem to be responsible for both the differences in the response patterns and the time frames.

The apparent key role which *intensity* plays in modifying the ^{3}H-leucine incorporation is further underscored by the finding that the elevated incorporation is more likely to occur in the sprint group than following endurance exercise (Table VI). It is possible that the trigger mechanism(s) responsible for signalling the initiation of the protein synthetic process in skeletal muscle is stimulated to a greater extent by the higher intensity sprint work.

Finally, the time when the rate of protein synthesis returns to pre-exercise levels differs between the endurance and sprint groups. Following the acute endurance exercise, the ^{3}H-leucine incorporation into protein returned to pre-exercise control values within 48 hours post exercise.

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Following the acute sprint exercise, the protein synthetic activity had returned to pre-exercise control values within 60 hours in all animals. This difference in the time where pre-exercise levels are re-established following sprint or endurance work has implications for training and will be discussed further in the section which deals with the practical implications of this study.

C. TRANSFER RNA - PROTEIN SYNTHESIS RELATIONSHIP

The data for the tRNA labelling by ^{3}H-leucine and ^{3}H-leucine incorporation into protein indicates that tRNA is modified in the post-exercise response of muscle protein metabolism.

When the levels of ^{3}H-leucine incorporation into protein are elevated, the levels of ^{3}H-leucyl-tRNA are relatively low (Tables VIII to XI), indicating that aminoacylation of tRNA is not rate limiting in the sequence of reactions of protein synthesis. Another explanation for the observed relationship between tRNA and protein synthesis pre-supposes a stimulated increase in tRNA turnover in some animals during the observed post-exercise response ranges. This increased turnover may be partially responsible for the observed increases in protein synthetic activity in those animals. When the level of ^{3}H-leucyl-tRNA is high, the incorporation of ^{3}H-leucine into protein is low. This would suggest that a greater proportion of the radio-labelled amino acids remain bound to the tRNA molecule (an increased

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charging level of tRNA associated with a decrease in turnover rate) and have not been made available to the polyribosomal protein synthetic apparatus. On the other hand, when the level of ^{3}H-leucyl-tRNA is low, the level of ^{3}H-leucine incorporated into new protein is high. This could reflect an increase in the turnover rate of leucyl-tRNA where a greater proportion of tritiated amino acids are made available to the protein synthetic system, and resultant increases in the levels of ^{3}H-leucine incorporated into protein are seen.

It is outlined elsewhere in this report (p.83) that the the synthesis of protein is dependent upon the interactions of RNA and DNA. As there now is some evidence suggesting that tRNA does play a role and can be modified in the protein metabolic response to acute exercise, the question arises as to the role that mRNA and rRNA might play in shaping this adaptive response. The results of this study, because of changes in the level of aminoacylation of tRNA, would indicate that the trigger mechanism(s) which initiate(s) an increased activity in the sequence of events leading to the formation of new protein, is operating at, or prior to, the level of *translation*. Whether or not the *transcription* of the coded message locked into the DNA structure, the *translocation* of the newly formed mRNA from the nucleus of the cell to the cytoplasm, the binding of the mRNA polymers to the ribosomal protein, or the *translation* of the message carried by mRNA (involving the aminoacylated

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tRNA) are affected, is still unknown. Further investigation is required to elucidate the subcellular mechanisms involved in the protein metabolic adaptation to exercise. Once this has been achieved, we may better understand how to modify the training stimulus in order to achieve a desired training adaptation.

In summary, it can be concluded as follows;

- The protein synthetic response as a result of acute exercise shows a pattern where elevated ^{3}H-leucine incorporation into protein is seen from 12 to 36 hours following endurance work and at both 0 time and between 24 and 48 hours following sprint work. The cause of the different responses between endurance and sprint exercise is unknown. Furthermore, all fractions appear to respond in the same manner and pre-exercise ^{3}H-leucine incorporation into protein levels are re-established within 48 hours following endurance exercise and within 60 hours following sprint work.
- The changes in protein synthesis in response to exercise may not follow the same time course in all animals. Although the physical intensity of the exercise bout can be calculated, the *effective stress* on each animal is difficult to quantify. This relative intensity could be responsible for the differences seen in the time-course of response of animals to a specific exercise stimulus.
- Finally, leucyl-tRNA levels are modified in response to exercise. This would indicate that part of the mechanism

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by which the training effect is built in response to exercise overload may involve modification of the *translation* step in protein synthesis.

D. PRACTICAL IMPLICATIONS

The data suggest that not all animals respond in the same manner to a set exercise stimulus. This observation underscores the already well recognized need for individuality in training programs. Because the individual response to an exercise type and load may be different, there must be some attempt made to evaluate individual response patterns. Further investigation into the protein metabolic response following acute exercise may eventually elucidate the mechanism responsible for the sequence of events which lead to adaptation of the training effect. When this is achieved, the training stimulus may be manipulated and monitored more effectively on an ongoing basis.

Elevated ^{3}H-leucine incorporation into protein was evident 12 to 36 hours following endurance exercise and at both 0 time and between 24 and 48 hours following sprint exercise. The implications of these findings lie, not in the specific response times themselves (because of species differences, results found in the rat do not necessarily reflect that response which would be seen in the human), but in the fact that differences are seen in the response of muscle metabolism to sprint or endurance exercise. It appears that the high intensity intermittent exercise

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results in a greater percentage of elevated responses and a bimodal response pattern when compared to endurance exercise. Associated with these findings are the times following exercise where the ^{3}H-leucine incorporation into protein returns to pre-exercise levels (*ie.* endurance - within 48h; sprint - within 60h). These data suggest the need for greater recovery time following high intensity intermittent sprint work than following endurance exercise.

The exercise schedule outlined in the present investigation involved work of a sprint or endurance nature on each of two consecutive days. How protein metabolism responds in a situation where two exercise sessions are performed on the same day, or exercise sessions of differing intensity and duration are performed on three consecutive days...or on four, as yet is to be determined. Whether an opportunity has been given for the training effect to be built prior to an additional stress being imposed on those systems is still unknown. These are just several of the issues which must be dealt with before the full implications of the effect of different types and amounts of exercise on protein metabolism in skeletal muscle can be realized.

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APPENDIX A

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APPENDIX A

E. REVIEW OF LITERATURE

SKELETAL MUSCLE FIBER TYPES AND THE NOTION OF RECRUITMENT

As myofibrillar ATPase activity has been shown to be closely related to contractile speed (Barany *et al.*, 1965), the two major fiber type categories histochemically distinguished on this basis can be designated as consisting of either slow-twitch (low levels of myofibrillar ATPase activity) or fast-twitch (high levels of myofibrillar ATPase activity) fibers (Gollnick *et al.*, 1972a, 1972b; Barnard *et al.*, 1971). Because of the metabolic and contractile characteristics of these two pools of muscle fibers, the terms slow-twitch and slow-oxidative (SO) are often used synonymously, as are the terms fast-twitch and fast-glycolytic (FG). Although this specific two fiber profile has been shown to exist in human skeletal muscle, much experimental evidence supports the fact that other mammalian skeletal muscle (prosimian, rat, mouse, etc.) is composed of a third distinct muscle fiber type (Edgerton and Simpson, 1969; Armstrong *et al.*, 1974, 1975; Khan *et al.*, 1974; Gillespie *et al.*, 1974) which has been termed fast-oxidative-glycolytic (FOG). These fibers show both high levels of myofibrillar ATPase activity (Burke and Edgerton, 1975) as well as heavy staining for oxidative enzyme potential (Peter *et al.*, 1972). This particular fiber type

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classification system (SO, FG, FOG) was proposed by Peter and co-workers (1972).

Thus it would appear that mammalian skeletal muscle is composed of fibers which are characterized by differences in contractile, metabolic and functional parameters (reviewed by Close, 1972). This distinction between fast-twitch and slow-twitch fibers with differing enzymatic and structural characteristics forms the basis of the idea or concept of motor unit (*ie.* specific fiber) recruitment.

Furthermore, the findings of Henneman and Olson (1965), Grimby and Hannerz (1968), Hannerz (1974) and Tanji and Kato (1973) with electromyographic recordings of single motor units would seem to indicate the presence of metabolically and characteristically different muscle fiber pools which can be selectively called into play in the performance of a particular type and intensity of work, and, in part, seem to be regulated by higher center input (Hannerz and Grimby, 1973).

Histochemical evidence (in particular, analysis of glycogen depletion patterns) also suggests that the mechanism postulated by Henneman and Olson (1965) exists where, at low to moderate stimulation intensity and frequency, those fibers with the smallest motorneurons (SO, FOG) seem to be predominantly recruited, and where those fibers with the largest motorneuron size (FG) are called into play only at the higher intensities of stimulation. Support for this hypothesis comes from other authors who

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have shown that the effect of exercise on the glycogen store is dependent on the work load employed (Hultman *et al.*, 1971; Saltin and Karlsson, 1971) and from the analysis of glycogen depletion patterns in exercising rats (Armstrong *et al.*, 1974, 1975; Edgerton and Simpson, 1969), prosimians (Gillespie *et al.*, 1974), guinea pigs (Edgerton and Simpson, 1969), and humans (Piehl, 1974; Edgerton *et al.*, 1975; Gollnick *et al.*, 1973a, 1973b, 1974a, 1974b; Costill *et al.*, 1973).

PROTEIN TURNOVER, OVERLOAD AND EXERCISE

The mechanisms involved in the synthesis of protein are well understood and have been shown to be dependent upon the interactions of the nucleic acids, DNA and RNA (Lehninger, 1970). Thus, the transcription and translation of the coded messages locked into the DNA structure are achieved by such enzymes as RNA polymerase and the actions of messenger, transfer and ribosomal RNA. These processes result in the synthesis of proteins (either structural of enzymatic) by means of peptide bond formation and the addition of amino acid residues to the newly manufactured protein (Lehninger, 1970). The metabolism of protein, however, must not be considered solely in terms of synthesis, but instead as a shifting balance between the processes of synthesis and degradation (Young, 1970; Burleigh, 1974; Millward *et al.*, 1970a, 1970b, 1975). During the process of growth, a shift in this balance exists towards the synthesis of new protein (Millward *et al.*, 1975; Srivastava and Chardhary, 1969;

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Young, 1970; Burleigh, 1974) and, in effect, muscle hypertrophy and increased metabolic function has been shown to be accompanied by a net gain in protein synthesis (Young, 1970, 1974). Likewise, in a review by Goldberg and co-workers (1975), increases in muscle weights, DNA, RNA, protein content and turnover, fiber areas and amino acid uptake were demonstrated in skeletal muscle as a result of compensatory adaptation (induced by tenotomy of the Achilles tendon). In this situation, the synergist muscles, soleus and plantaris, must assume the work load normally handled by the gastrocnemius muscle. Whether or not the changes induced in response to this protocol reflect an increased protein synthesis, a decreased protein degradation or some combination of these two factors is equivocal. Despite the question as to how physiological this situation might be, it has been suggested that it is the increased tension development which is the critical event in initiating this compensatory hypertrophy (Goldberg, 1975).

Shifts in the protein metabolic balance have also been demonstrated as a result of physical exercise and training (Gordon *et al.*, 1967a, 1967b; Hubbard *et al.*, 1974; Jaweed *et al.*, 1974; Dohm *et al.*, 1977a, 1977b). As the neural recruitment of specific muscle fibers dictates the speed at which the whole muscle will contract (Barany, 1967; Close, 1972), the substrates it will use (Baldwin *et al.*, 1973; Pernow *et al.*, 1971) and the enzymatic pathways it will employ to supply the required energy to do the work

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(Edington *et al.*, 1973; Holloszy *et al.*, 1973, 1975a, 1975b; Mole *et al.*, 1971), it can be said that the physical work capacity of an organism or group of muscles may be dependent upon it's contractile protein (myosin-actin) and its ability to produce the energy needed for that contraction. Evidence from physical training studies does support this notion of protein adaptation in working muscle (Helander, 1961; Gordon *et al.*, 1967a, 1967b; Holloszy *et al.*, 1973, 1975a, 1975b) and Maxwell and his co-workers (1971) hypothesized that this phenomenon of selective adaptation in muscle reflected the specific recruitment of those fibers (in this particular study, with respect to endurance training).

However, the evidence relating to the specificity of protein adaptation as a result of certain types of training is somewhat controversial. Helander (1961), with endurance trained guinea pigs (4 months on a running program), demonstrated an increase in myofibrillar protein in the exercised animals as compared to their unexercised controls. However, Hubbard *et al.* (1974), again with endurance training, showed a non-selective change of sarcoplasmic, myofibrillar and stromal proteins. On the other hand, the opposite training effects were demonstrated by Gordon *et al.* (1967a) and Jaweed *et al.* (1974) with endurance running and swimming programs. Here, increases in sarcoplasmic proteins (mitochondrial proteins) and decreases in myofibrillar proteins (contractile proteins) were seen in rat quadriceps and gastrocnemius muscles. These findings have been

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substantiated by others (Yakovlev *et al.*, 1963). As well, Gordon *et al.* (1967b) and Jaweed *et al.* (1974), using rats trained by weight lifting, have demonstrated selective increases in myofibrillar proteins with little change in sarcoplasmic components. This increase in myofibrillar protein was also associated with selective hypertrophy of FG fibers (those presumably recruited) but not of SO or FOG fibers (Gordon *et al.*, 1967b).

Similarly, Gordon and co-workers (1967a), with endurance training, showed no net change in whole muscle size but demonstrated an increase in the fiber areas of the red (SO,FOG) fiber types. Faulkner *et al.* (1971) and Maxwell *et al.* (1973), studying guinea pigs, discovered an increase in the mean area of SO fibers in soleus muscles along with hypertrophy of all three fiber types in plantaris, and an increased proportion of red to white muscle fibers in plantaris muscles after an endurance training period. In effect, fiber composition alterations in selected muscles have been demonstrated with both endurance (Edgerton *et al.*, 1973; Barnard *et al.*, 1970; Faulkner *et al.*, 1971; Maxwell *et al.*, 1971, 1973; Syrovy *et al.*, 1972; Wilkinson *et al.*, 1976) and sprint (Saubert *et al.*, 1973; Mackie, 1976; Wilkinson *et al.*, 1976) training. These fiber alterations have not, however, been demonstrated in all training studies (Bagby *et al.*, 1972; Exner *et al.*, 1973a, 1973b; Fitts *et al.*, 1973, 1974) and it has been suggested that the changes demonstrated in these studies may have been confounded by

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developmental changes (Mackie, 1976; Wilkinson *et al.*, 1976). In fact, some of the controversial evidence cited earlier pertaining to the specificity of protein synthesis may be due to a similar interaction of training response and developmental changes in prepubertal and mature animals. This increase in protein synthesis with exercise was reflected in a more general way by Hubbard *et al.* (1974) who showed that there was more protein in exercised as compared to control animals, although the body weights of the animals were the same.

On a more enzymatic level, the specificity of protein adaptation to a specific exercise stress is well documented. In general, training results in an increase in the ability to supply energy to the contactile apparatus (Wenger and Reed, 1976) as well as changes in the concentration and activities of enzymes of intermediary metabolism (Bass *et al.*, 1969; Staudte and Pette, 1972). More specificaly, increases in oxidative enzyme capacity have been demonstrated with endurance exercise and training (Barnard *et al.*, 1970, 1971; Fitts *et al.*, 1974; Edington *et al.*, 1970, 1973; Gollnick *et al.*, 1972; Peter *et al.*, 1972; Terjung *et al.*, 1972; Holloszy *et al.*, 1973, 1975a, 1975b) and, isometric or sprint training seems to alter glycolytic as well as some oxidative enzymes (Bagby *et al.*, 1972; Exner *et al.*, 1973a, 1973b; Saubert *et al.*, 1973; Staudte *et al.*, 1973; Thorstensson *et al.*, 1975) to result in an increase in the metabolic capacity for energy production (Saltin, 1973;

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Holloszy, 1975a).

Analysis of nucleic acid content in trained skeletal muscle gives rise to more controversial findings. Gordon and co-workers (1967a, 1967b) found no change in total muscle DNA content from animals trained by swimming, endurance running or weight lifting whereas Bailey *et al.* (1973), with animals training by means of a swimming program, showed a significant difference between exercise and control animals in DNA concentration (μg/mg tissue) at 12 weeks of age, and in the protein:DNA ratio at 6 weeks of age. In contrast, Hubbard *et al.* (1974) showed changes in DNA content, as a result of an endurance running program, only in selective muscles. Thus, the gastrocnemius muscle showed no change in DNA content while significant decreases in DNA concentrations of soleus and plantaris muscles were seen in rats exercised up to 18 weeks of age. It is possible that these discrepancies again may be age related, as the animals used in these particular studies were from different age groups (Hubbard *et al.*, 1974; Bailey *et al.*, 1973).

RNA concentrations in rat cardiac and skeletal muscle have also been studied after acute exhaustive swimming (Bostrom *et al.*, 1974). Levels of RNA in cardiac tissue were significantly decreased immediately after the exercise bout whereas no change was seen in RNA concentration in the gastrocnemius muscle. However, after one to three days post-exercise, levels of RNA were significantly higher in both gastrocnemius and cardiac muscles. It was suggested by

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Bostrom and his co-workers (1974) that the decline in cardiac muscle RNA following the exercise bout reflected an increase in protein catabolism - a shift in the balance towards degradation. Subsequent to this initial catabolic period (24 - 72 hrs.) an increase in RNA mediated protein synthesis was seen to occur (Bostrom *et al.*, 1974) and has since been confirmed in a review of Soviet research by Rogozkin (1976) and of prolonged endurance exercise by Poortmans (1975).

Evidence of a more indirect nature (study of urinary nitrogen levels) involving very intense physical exercise also supports the notion of enhanced protein storage and synthesis with strenuous exercise accompanied by little change in the protein breakdown (Consolazio *et al.*, 1975).

The incorporation of radioactively tagged amino acids into newly synthesized protein has also been studied in a limited way. Pain and Manchester (1970) examined the incorporation of a radioactively tagged amino acid into electrically stimulated rat skeletal muscle and found that it was decreased during and immediately following stimulation. A subsequent increase in amino acid uptake into protein was then observed several hours after stimulation. Similar results with tritiated Uridine have been reported by Muchnick and Kotsiam (1975) in electrically stimulated gastrocnemius muscle. Dohm *et al.* (1977a) examined incorporation of ^{14}C tagged leucine into protein in response to endurance training and discovered that there was a

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suppression of tagged leucine incorporation (3 hours post-exercise) into the stromal fraction but no change in sarcoplasmic or myofibrillar components. These findings seem to support the notion of specificity and, one might hypothesize that if these authors had followed the tagged leucine incorporation for a number of hours post-exercise (24 to 72 hrs.) they might perhaps have discovered (as Pain and Manchester did (1970)) an increase in the incorporation of the tagged leucine into protein in that fraction. Evidence from other work conducted by Dohm *et al*. (1977b) indicates that during exercise, the balance in protein metabolism shifts towards catabolism and, it is suggested that the increased amino acid oxidation could serve some, as yet undefined, role in energy production for the work intended.

Finally, McManus *et al*. (1975) with endurance trained guinea pigs, demonstrated that the training effect on tritiated leucine incorporation was significant only in sarcoplasmic (vs. myofibrillar) proteins of animals with normal gonadal function. The authors suggested that the level of physical activity of the young animals studied appeared to be more important than gonadal endocrine function (testosterone) in altering protein metabolism and muscle and body weights.

Thus, the notion of specificity of protein adaptation associated with selective recruitment of individual skeletal muscle fiber types (and fractions within those fibers) would

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seem to have some support. A further examination of this phenomenon and an analysis of the time course over which these changes might occur would be of benefit to help elucidate the mechanisms involved in the acute, and possibly long term, response of protein adaptive systems to physical exertion.

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APPENDIX B

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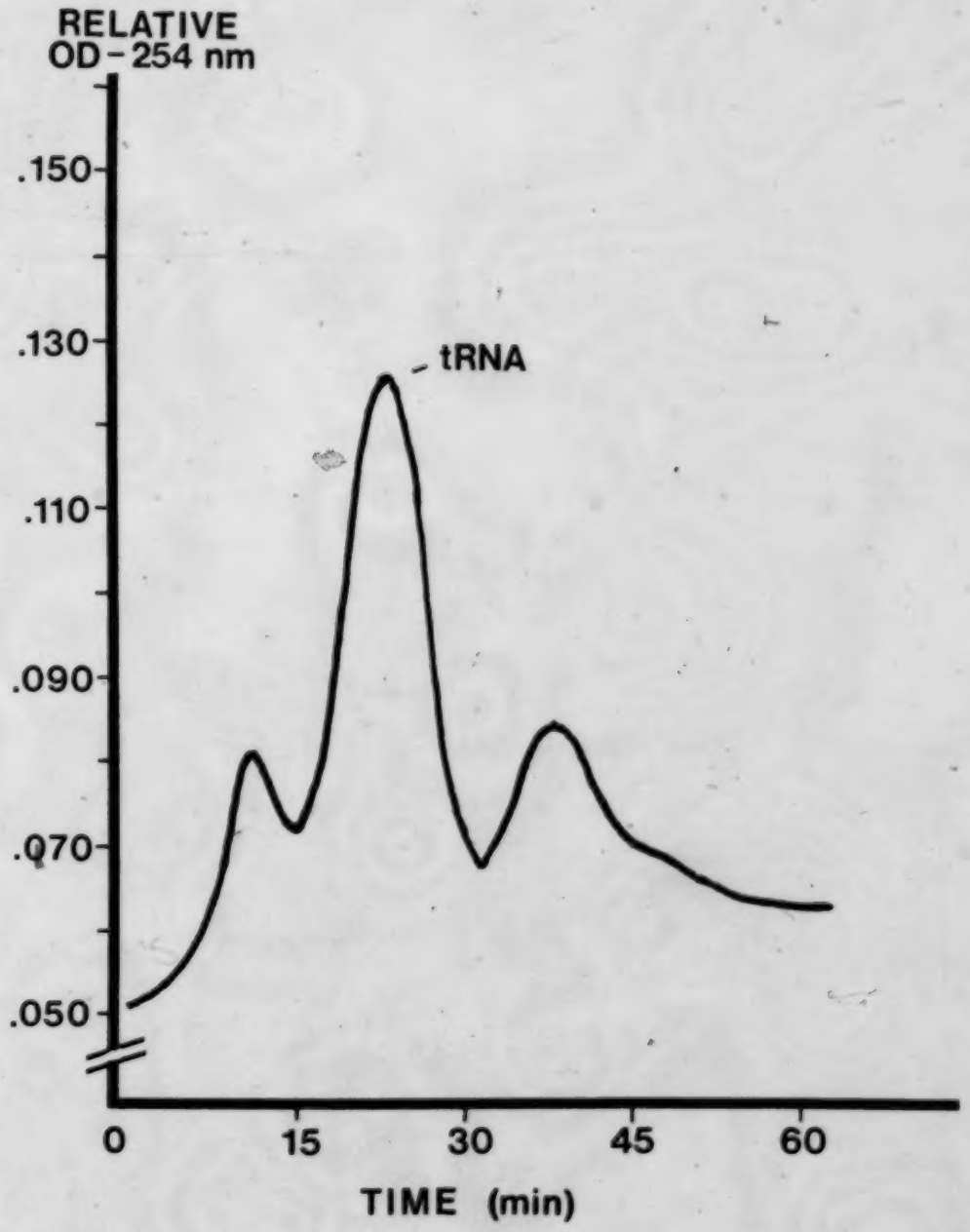

Figure 18. Sample chromatograph of eluted RNA used to distinguish tRNA peak. (flow rate - 20.0 cm/h; wavelength - 254nm; buffer - 0.1M sodium phosphate (pH 7.0); paper speed - 1in/h)

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Table XIV. Method of calculation of 260:280nm wavelength ratio used to identify the peak in the eluted sample characteristic of transfer RNA.

OD260 nm	OD280 nm	260 -blank	280 -blank	ratio 260/280	/20	cpm	cpm -blank	S.A. dpm/OD260
.055	.051	.002	.002	1.00				
.054	.050	.001	.001	1.00				
.057	.054	.004	.005	0.80				
.073	.063	.020	.014	1.43				
.072	.061	.019	.012	1.58				
.102	.076	.049	.027	1.82	.0025	270	250	100000*
.123	.085	.070	.036	1.94	.0035	324	304	86857*
.098	.073	.045	.024	1.86	.0023	193	173	75217*
.070	.058	.017	.009	1.89				
.075	.056	.022	.007	3.14	.0011	83	63	57217
.134	.070	.081	.021	3.86	.0041	130	110	26829
.140	.071	.087	.022	3.96	.0044	143	123	27955
.104	.061	.051	.012	4.25				
Mean =				1.87				87358

blank - .053
.049

Animal used was AE.ACC. #2 (Plantaris)
* - eluted samples used for calculation of tRNA S.A.

$$\text{Equation} - \frac{\text{cpm - blank}}{\text{OD260 - blank / 20}} \times 2.913 = \text{S.A.(dpm/OD260)}$$

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Table XV. Example of the calculation of protein specific activity (dpm/mg protein) for animal #2 of the sedentary control group.

Frac	M	Protein OD260nm	-bl*	pr1 mg.	pr2 mg.	Counts cpm	-bl*	S.A. cpm	S.A. dpm
TOT	S	.206-.210	.111	.2362	.7085	2614-3326	2938	4147	12080
	P	.210-.208	.112	.2383	.7149	1966-3142	3022	4227	12313
MYO	S	.665-.573	.522	1.111	3.332	3811-3765	3756	1127	3283
	P	.377-.373	.278	.5915	1.775	3502-3514	3476	1959	5707
MIT	S	.232-.227	.133	.2830		222- 308	233	823	2397
	P	.173-.176	.078	.1660		157- 146	120	723	2106
SOL	S	.175-.175	.078	.1660		378- 499	407	2452	7143
	P	.236-.229	.136	.2894		397- 482	408	1410	4107

Biuret Blank =.097-.096 Biuret Factor = 0.042

Counts Blank = 28 - 35

*(-bl = mean of two previous values minus value of blank)

pr1 - Amount of Protein in a 0.1 ml aliquot of sample

pr2 - Amount of Protein in a 0.3 ml aliquot of sample

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Table XVI. Serum counts (dpm) measured in a 20 µl sample of blood taken from experimental animals (A1,A2 - aliquots).

Group	Animal#	A 1	A 2	Group	Animal#	A 1	A 2
SED.C.	1	13900	14536	SED.C.	1	13900	14536
	2	12265	13256		2	12265	13256
	3	14087	14764		3	14087	14764
AN.ACC.	1	11859	12588	AE.ACC.	1	9744	10401
	2	10470	10821		2	11314	11416
	3	13813	12347		3	12065	13380
AN. 0	1	11871	10490	AE. 0	1	12713	13547
	2	13673	14187		2	11943	12966
	3	14007	15386		3	13000	14457
	4	11178	12800				
AN. 12	1	12229	12894	AE. 12	1	8822	9405
	2	11417	12153		2	13876	13143
	3	10120	10863		3	10884	12094
AN. 24	1	14300	15385	AE. 24	1	11617	12382
	2	12065	12900		2	9876	11550
	3	14579	16100		3	10958	11711
	4	13069	12869				
AN. 36	1	12869	13527	AE. 36	1	10355	11079
	2	12424	13338		2	12931	14160
	3	11032	11824		3	9706	9206
AN. 48	1	13640	15057	AE. 48	1	10370	11122
	2	11142	12135		2	12400	12831
	3	10435	11128		3	11583	13194
	4	16508	17867				
AN. 60	1	11711	11188	AE. 60	1	12866	14357
	2	11811	11005		2	11422	10521
	3	11194	12176		3	11543	12192
AN. 72	1	11744	12756	AE. 72	1	8852	9586
	2	16546	15200		2	11375	12188
	3	11489	12713		3	11573	12847

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Table XVII. Body weights(g) of experimental animals at time of sacrifice.

Group	Animal #	Body Wt.	Group	Animal #	Body Wt.
SED.C	1	319	SED.C.	1	319
	2	315		2	315
	3	303		3	303
AN.ACC.	1	219	AE.ACC.	1	232
	2	313		2	232
	3	287		3	276
AN. 0	1	252	AE. 0	1	270
	2	261		2	248
	3	269		3	258
	4	250			
AN. 12	1	236	AE. 12	1	211
	2	290		2	249
	3	238		3	228
AN. 24	1	208	AE. 24	1	243
	2	258		2	235
	3	270		3	227
	4	256			
AN. 36	1	259	AE. 36	1	238
	2	233		2	229
	3	229		3	257
AN. 48	1	247	AE. 48	1	243
	2	242		2	236
	3	188		3	268
	4	252			
AN. 60	1	206	AE. 60	1	261
	2	243		2	258
	3	237		3	215
AN. 72	1	260	AE. 72	1	233
	2	221		2	262
	3	216		3	222

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Table XVIII. Muscle weights (mg) for all experimental animals (S - soleus; P - plantaris).

GROUP	ANIMAL#	MUSCLE S RL	S LL	P RL	P LL	GROUP	ANIMAL#	MUSCLE S RL	S LL	P RL	P LL
SED.C.	1	126	140	308	318						
	2	151	152	290	278						
	3	131	99	239	254						
AN.ACC.	1	104	99	204	203	AE.ACC.	1	97	112	232	234
	2	130	147	317	326		2	104	115	219	235
	3	104	133	272	258		3	116	123	250	258
AN. 0	1	107	119	230	236	AE. 0	1	110	95	271	268
	2	112	138	240	260		2	106	119	246	229
	3	128	100	280	252		3	132	124	241	242
	4	120	89	227	211						
AN. 12	1	108	125	238	237	AE. 12	1	123	114	203	190
	2	145	145	272	278		2	106	134	258	272
	3	120	126	225	231		3	119	122	254	251
AN. 24	1	78	84	209	189	AE. 24	1	130	115	244	265
	2	115	100	238	254		2	117	120	223	211
	3	128	137	263	236		3	109	108	214	220
	4	145	136	251	250						
AN. 36	1	113	102	247	252	AE. 36	1	100	127	216	228
	2	103	111	183	191		2	125	126	239	245
	3	99	119	231	236		3	138	128	256	258
AN. 48	1	123	133	228	227	AE. 48	1	115	122	231	228
	2	113	116	230	244		2	109	114	240	247
	3	94	83	160	156		3	121	129	258	258
	4	125	105	244	235						
AN. 60	1	93	95	223	212	AE. 60	1	131	147	240	252
	2	102	88	252	245		2	117	129	262	263
	3	105	116	204	209		3	102	108	222	222
AN. 72	1	107	116	244	252	AE. 72	1	120	124	242	143
	2	96	117	195	210		2	130	135	251	244
	3	119	105	202	191		3	97	114	204	225

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APPENDIX C

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Table XIX. Incorporation of the radionuclide L-(4,5 ^{3}H)-leucine (dpm/mg protein) in all fractions (total homogenate -TOT, myofibrillar-nuclear - MYO, mitochondrial - MIT and soluble - SOL) of soleus (S) and plantaris (P) muscles from animals in the endurance group.

GROUP	FRACTION	MUSCLE	ANIMAL NUMBER 1	2	3	4	MEAN
SED.C.	TOT	S	17079	12080	11422		13527
		P	13152	12313	10318		11928
	MYO	S	8672	3283	4046		5334
		P	6936	5707	4710		5784
	MIT	S	7454	2397	1934		3928
		P	3499	2106	1646		2417
	SOL	S	6120	7143	5118		6127
		P	3350	4107	2965		3474
AE.ACC.	TOT	S	2278	2377	2418		2358
		P	1745	2022	1896		1888
	MYO	S	2054	1497	2109		1887
		P	1643	1891	1628		1721
	MIT	S	1987		973		1480
		P	1512		4000		2756
	SOL	S	3309	3458	3397		3388
		P	1943	2648	2234		2275
AE. 0	TOT	S	1646	1920	2269		1945
		P	1847	1474	1727		1683
	MYO	S	912	1369	1241		1174
		P	1404	1250	1413		1356
	MIT	S	1221	1459	1445		1375
		P	1797	1317	1669		1594
	SOL	S	2811	3673	3740		3408
		P	2092	1920	2220		2077

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Table XIX (cont'd)... Incorporation of the radionuclide L-(4,5 ^{3}H)-leucine (dpm/mg protein) in all fractions (total homogenate -TOT, myofibrillar-nuclear - MYO, mitochondrial - MIT and soluble - SOL) of soleus (S) and plantaris (P) muscles from animals in the endurance group.

GROUP	FRACTION	MUSCLE	ANIMAL NUMBER 1	2	3	4	MEAN
AE. 12	TOT	S	92400	2730	2983		32704
		P	27848	2022	2540		10803
	MYO	S	74925	2173	2406		26501
		P	24181	1655	2496		9444
	MIT	S	81774	2572	2362		28903
		P	15040	1267	1966		6091
	SOL	S	131589	3796	4107		46497
		P	36174	2826	3021		14007
AE. 24	TOT	S	174227	84503	3350		87360
		P	29398	14239	2636		15424
	MYO	S	118285	77768	2427		66160
		P	24399	10775	2074		12416
	MIT	S	92861	86770	2424		60685
		P	2231	10117	1320		4556
	SOL	S	279593	123357	1116		134689
		P	40756	17385	862		19668
AE. 36	TOT	S	3609	95217	993		33273
		P	2526	10032	667		4408
	MYO	S	2351	58353	734		20479
		P	2118	7035	621		3258
	MIT	S	1390	37747	2817		13985
		P	1760	5375	1637		2924
	SOL	S	4352	147165	5054		52190
		P	3228	13738	2517		6494

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Table XIX (cont'd)... Incorporation of the radionuclide L-(4,5 ^{3}H)-leucine (dpm/mg protein) in all fractions (total homogenate -TOT, myofibrillar-nuclear - MYO, mitochondrial - MIT and soluble - SOL) of soleus (S) and plantaris (P) muscles from animals in the endurance group.

GROUP	FRACTION	MUSCLE	ANIMAL NUMBER 1	2	3	4	MEAN
AE. 48	TOT	S	2301	3140	3956		3132
		P	1934	2438	2965		2446
	MYO	S	1716	1888	3108		2237
		P	1707	1943	2144		1931
	MIT	S	1524	1162	3376		2021
		P	1442	1416	1748		1535
	SOL	S	3099	3333			3216
		P	2429	2441	1328		2066
AE. 60	TOT	S	2636	2695	3228		2853
		P	1809	2068	2488		2122
	MYO	S	1649	1707	1896		1751
		P	1611	1701	1902		1738
	MIT	S	1492	1553	1506		1517
		P	1532	1599	1398		1510
	SOL	S	3740	3726	4498		3988
		P	2380	2607	3108		2698
AE. 72	TOT	S	2217	2258	2447		2307
		P	2229	1585	1789		1868
	MYO	S	1573	1675	1981		1743
		P	1975	1366	1617		1653
	MIT	S	1063	1940	2872		1958
		P	1815	1104	1701		1540
	SOL	S	3021	3030	3155		3035
		P	2628	2240	2383		2417

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Table XX. Incorporation of the radionuclide L-(4,5 ^{3}H)-leucine (dpm/mg protein) in all fractions (total homogenate -TOT, myofibrillar-nuclear - MYO, mitochondrial - MIT and soluble - SOL) of soleus (S) and plantaris (P) muscles from animals in the sprint group.

GROUP	FRACTION	MUSCLE	ANIMAL NUMBER 1	2	3	4	MEAN
SED.C.	TOT	S	17079	12080	11422		13527
		P	13152	12313	10318		11928
	MYO	S	8672	3283	4046		5334
		P	6936	5707	4710		5784
	MIT	S	7454	2397	1934		3928
		P	3499	2106	1646		2417
	SOL	S	6120	7143	5118		6127
		P	3350	4107	2965		3474
AN.ACC.	TOT	S	20697	2904	1803		8468
		P	22529	2654	1130		8771
	MYO	S	12584	1923	1157		5221
		P	16747	2129	976		6617
	MIT	S	19549	1995			10772
		P	13752	2083			7918
	SOL	S	34059	4949	2360		13789
		P	32614	3720	1486		12607
AN. 0	TOT	S	2307	2042	124950	109404	59676
		P	1829	1451	27184	11195	10415
	MYO	S	1783	1582	87073	67745	39546
		P	1541	1323	19878	9383	8031
	MIT	S	2482	816	96237	69991	42382
		P	1827	1017	23758	13473	10019
	SOL	S	3834	2928	202098	171684	95136
		P	2523	2097	39095	16593	15077

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Table XX (cont'd)... Incorporation of the radionuclide L-(4,5 ^{3}H)-leucine (dpm/mg protein) in all fractions (total homogenate -TOT, myofibrillar-nuclear - MYO, mitochondrial - MIT and soluble - SOL) of soleus (S) and plantaris (P) muscles from animals in the sprint group.

GROUP	FRACTION	MUSCLE	ANIMAL NUMBER 1	2	3	4	MEAN
AN. 12	TOT	S	2534	3134	2660		2777
		P	2089	2112	2010		2070
	MYO	S	1707	2491	1975		2058
		P	1410	1765	1771		1649
	MIT	S	2167	1937	1725		1943
		P	1882	1684	1535		1700
	SOL	S	3880	4582	3784		4082
		P	2890	2543	3105		2846
AN. 24	TOT	S	47342	1803	2243	95051	36610
		P	6709	1497	1911	54004	16030
	MYO	S	30368	1075	1492	47829	20191
		P	5389	883	1346	45087	13176
	MIT	S	63064	1168	1602	54036	29968
		P	9301	1049	1462	42204	13504
	SOL	S	70419	2814	3504	165193	60680
		P	12418	2080	2791	73681	22743
AN. 36	TOT	S	92505	54799	61922		69742
		P	10123	16365	37190		21226
	MYO	S	72333	33430	42489		49417
		P	8087	9712	30284		16021
	MIT	S	69230	58583	64013		63942
		P	7443	11940	17997		12460
	SOL	S	138403	84902	85960		103088
		P	2039	25707	54083		27276

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Table XX (cont'd)... Incorporation of the radionuclide L-(4,5 ^{3}H)-leucine (dpm/mg protein) in all fractions (total homogenate -TOT, myofibrillar-nuclear - MYO, mitochondrial - MIT and soluble - SOL) of soleus (S) and plantaris (P) muscles from animals in the sprint group.

GROUP	FRACTION	MUSCLE	ANIMAL NUMBER 1	2	3	4	MEAN
AN. 48	TOT	S	3298	3784	93589	122530	55800
		P	3009	3525	22780	19284	12150
	MYO	S	1961	2526	62734	87457	38670
		P	2290	2389	15789	15925	9098
	MIT	S	1159	2630	105625	109605	54755
		P	2243	3365	19074	14798	9870
	SOL	S	4929	4990	180236	181757	92978
		P	3700	4387	34743	27505	17584
AN. 60	TOT	S	3414	2575	3233		3074
		P	2403	2217	2741		2454
	MYO	S	1981	1154	2010		1715
		P	1861	1582	1818		1754
	MIT	S	2028	950	2336		1771
		P	2118	1751	1894		1921
	SOL	S	4317	3807	4061		4062
		P	4090	3067	4361		3839
AN. 72	TOT	S	3120	2543	664		2109
		P	2502	1934	906		1781
	MYO	S	1818	1110	1206		1378
		P	1888	1363	1232		1494
	MIT	S	1413	862	961		1079
		P	1876	1620	1206		1567
	SOL	S	4506	3437	3528		3824
		P	3647	2395			3021

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APPENDIX D

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Table XXI. Calculation of the conversion factor used to change cpm to dpm. An internal standard of known radioactivity was used. Data from animal 1 (AE. 60) was used for standardization.

Fraction	Muscle	Original cpm	New cpm	Difference
TOT	S	390	36817	36427
		423	37177	36694
	P	433	36100	35667
		424	39533	39109
	H	590	37950	37360
		600	37567	36967
MYO	S	628	37817	37189
		648	36717	36069
	P	585	38250	37765
		600	38750	38150
	H	843	37467	36624
		841	38500	37659
MIT	S	129	37917	37788
		140	39383	39243
	P	124	39417	39293
		126	39367	39241
	H	264	39783	39519
		260	38950	38690
SOL	S	255	40160	39905
		259	38217	37958
	P	329	39983	39654
		304	38617	38313
	H	595	39467	38872
		602	38867	38265
SERUM		14914	51700	36786
		15279	58125	42846
BLANK		27	38517	38490
		28	37200	37172
			Mean =	38133

Internal Standard = 1.327×10^6 dpm/gm solution
100µl=0.0837gm solution
Radioactivity added to samples = 111,070 dpm/100µl
Conversion factor for cpm to dpm on Beckman LS 250 counter: 111,070 / 38,133 = 2.913

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BIURET TECHNIQUE*

Reagent Preparation

1.5g of $CuSO_4.5H_2O$ and 6.0g of $NaKC_4H_4O_6.4H_2O$ were mixed in approximately 500ml of distilled water.

300ml of 10% NaOH was added to the above solution with constant swirling

The solution was then diluted to 1.0l with distilled water and stored in a 'Teflon' bottle.

PROCEDURE

0.5ml of the above reagent was added to 0.1ml of sample.

The mixture was agitated and left to stand for 10min at room temperature.

The Optical Density was spectrophotometrically measured at a wavelength of 540nm and the value multiplied by the standard curve factor (0.042) established for the Biuret reagent. The results were expressed as mg of protein in 0.01ml of sample.

* Modified from Gornall, A.G., *et al.*, *Journal of Biological Chemistry* 177:751-66, 1949.

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Table XXII. Sacrifice time schedule for experimental animals.

Group	Animal #	Time of Sacrifice(hrs)	Group	Animal #	Timeof Sacrifice(hrs)
SED.C.	1	8:30			
SED.C.	2	11:30			
SED.C.	3	14:30			
AN.ACC.	1	14:00	AE.ACC.	1	16:30
AN.ACC.	2	8:00	AE.ACC.	2	10:30
AN.ACC.	3	7:30	AE.ACC.	3	7:30
AN. 0	1	17:30	AE. 0	1	11:00
AN. 0	2	12:30	AE. 0	2	14:00
AN. 0	3	8:30	AE. 0	3	15:30
AN. 0	4	13:30			
AN. 12	1	11:30	AE. 12	1	7:30
AN. 12	2	19:30	AE. 12	2	19:30
AN. 12	3	8:30	AE. 12	3	12:30
AN. 24	1	14:30	AE. 24	1	10:30
AN. 24	2	17:30	AE. 24	2	13:30
AN. 24	3	20:30	AE. 24	3	16:45
AN. 24	4	7:30			
AN. 36	1	11:30	AE. 36	1	7:30
AN. 36	2	23:30	AE. 36	2	19:30
AN. 36	3	19:30	AE. 36	3	21:30
AN. 48	1	11:00	AE. 48	1	10:30
AN. 48	2	17:30	AE. 48	2	13:30
AN. 48	3	20:30	AE. 48	3	16:30
AN. 48	4	17:00			
AN. 60	1	11:30	AE. 60	1	19:30
AN. 60	2	23:30	AE. 60	2	19:30
AN. 60	3	8:30	AE. 60	3	21:30
AN. 72	1	14:30	AE. 72	1	10:30
AN. 72	2	17:30	AE. 72	2	13:30
AN. 72	3	20:30	AE. 72	3	16:30

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